STIC-ILL 258/462

From: Turner, Sharon
Sent: Friday, July 23, 1999 5:33 PM
To: STIC-ILL
Subject: 09207649

Please RUSH w/ **PUB DATE**

Embo J. 1998 17(19):5805-10

Glover et al, Mol. Chaperones Life Cycle Proteins 1998:193-224, Eds Fink AL, Dekker NY, NY.

Mol. Cell Biol., 1997 17(5):2798-2805

Embo J 1996 15(12):3127-34

Biochem Soc. Trans. 1998 26(3):486-90

Curr Genet 1998 34(2):146-151

Amyloid 1998 5(2):79-89

Guideb Mol Chaperones Protein-Folding Catal. 1997, 249-51

Biol Chem 1997 378(12):1521-30

PNAS 1997, 94(25):13932-37

Science 1997 277(5324):381-83

PNAS 1997 94(13):6618

J. Biochem 1997, 121(2):179-88

Science 1996 273(5275):622-26

PNAS 1996 93(5):2065-70

PNAS 1998 95(6):3275-80

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NO

Thanks!
Sharon L. Turner, Ph.D.
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EYW
7/29

ASL-1105
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Fr m: Turner, Sharon
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Embo J. 1998 17(19):5805-10

Glover et al, Mol. Chaperones Life Cycle Proteins 1998:193-224, Eds Fink AL, Dekker NY, NY.

Mol. Cell Biol., 1997 17(5):2798-2805

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Biochem Soc. Trans. 1998 26(3):486-90

990727B918

Curr Genet 1998 34(2):146-151

Amyloid 1998 5(2):79-89

Guideb Mol Chaperones Protein-Folding Catal. 1997, 249-51

Biol Chem 1997 378(12):1521-30 98121249

PNAS 1997, 94(25):13932-37

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Science 1997 277(5324):381-83

PNAS 1997 94(13):6618

J. Biochem 1997, 121(2):179-88

Science 1996 273(5275):622-26

PNAS 1996 93(5):2065-70

PNAS 1998 95(6):3275-80

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Embo J. 1998 17(19):5805-10

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Mol. Cell Biol., 1997 17(5):2798-2805

ZGE-9907283289

Embo J 1996 15(12):3127-34

Biochem Soc. Trans. 1998 26(3):486-90

Curr Genet 1998 34(2):146-151

Amyloid 1998 5(2):79-89 Solvent

Guideb Mol Chaperones Protein-Folding Catal. 1997, 249-51

Biol Chem 1997 378(12):1521-30

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PNAS 1997, 94(25):13932-37

Science 1997 277(5324):381-83

PNAS 1997 94(13):6618

J. Biochem 1997, 121(2):179-88

Science 1996 273(5275):622-26

PNAS 1996 93(5):2065-70

PNAS 1998 95(6):3275-80

Thanks!

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STIC-ILL

Fr m: Turner, Sharon
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Subject: 09207649

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Embo J. 1998 17(19):5805-10

Glover et al, Mol. Chaperones Life Cycle Proteins 1998:193-224, Eds Fink AL, Dekker NY, NY.

Mol. Cell Biol., 1997 17(5):2798-2805

Embo J 1996 15(12):3127-34

Biochem Soc. Trans. 1998 26(3):486-90

Curr Genet 1998 34(2):146-151

Amyloid 1998 5(2):79-89

990727B913

Guideb Mol Chaperones Protein-Folding Catal. 1997, 249-51

Biol Chem 1997 378(12):1521-30

PNAS 1997, 94(25):13932-37

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Science 1997 277(5324):381-83

PNAS 1997 94(13):6618

J. Biochem 1997, 121(2):179-88 9724461 1

Science 1996 273(5275):622-26

PNAS 1996 93(5):2065-70

PNAS 1998 95(6):3275-80

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7/27 VDS

STIC-ILL

258/457

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Subject: 09207649

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Embo J. 1998 17(19):5805-10

Glover et al, Mol. Chaperones Life Cycle Proteins 1998:193-224, Eds Fink AL, Dekker NY, NY.

Mol. Cell Biol., 1997 17(5):2798-2805

QP552 M64F54

Embo J 1996 15(12):3127-34

Biochem Soc. Trans. 1998 26(3):486-90

Curr Genet 1998 34(2):146-151

Amyloid 1998 5(2):79-89

Guideb Mol Chaperones Protein-Folding Catal. 1997, 249-51

Biol Chem 1997 378(12):1521-30

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PNAS 1997, 94(25):13932-37

Science 1997 277(5324):381-83

PNAS 1997 94(13):6618

J. Biochem 1997, 121(2):179-88

Science 1996 273(5275):622-26

PNAS 1996 93(5):2065-70

PNAS 1998 95(6):3275-80

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Sharon L. Turner, Ph.D.
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AGL - 33
R.C 7/26 $2.97

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     FILE 'CAPLUS' ENTERED AT 16:16:34 ON 23 JUL 1999
L1         16782 S INHIB?(5A)(AGGREG? OR PLAQUE?)
L2           109 S L1 AND AMYLOID?
L3             3 S L2 AND YEAST
L4             0 S L2 AND EUKARYO?
L5             0 S L2 AND PROKARY?
L6         12870 S "SUP35" OR "URE3" OR "PRP" OR PRION OR ?AMYLOID?
L7           522 S L6 AND L1
L8             6 S L7 AND YEAST
L9             3 S L8 NOT L3
L10            1 S L7 AND EUKARY?
L11            0 S L10 NOT L8
L12          990 S YEAST AND (AGGREG? OR PLAQUE?)
L13           32 S L12 AND L6
L14           26 S L13 NOT L8
L15            3 S L14 AND INHIB?
L16            3 S L15 NOT L8
L17            3 S L16 NOT L3
```

protease resistance

L2 ANSWER 5 OF 10 MEDLINE
AN 1998019217 MEDLINE
DN 98019217 PubMed ID: 9353306
TI Prion protein aggregation reverted by low temperature in transfected cells
carrying a prion protein gene mutation.
AU Singh N; Zanusso G; Chen S G; Fujioka H; Richardson S; Gambetti P; Petersen R B
CS Division of Neuropathology, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio 44106, USA.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1997 Nov 7) 272 (45) 28461-70.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199712
ED Entered STN: 19980109
Last Updated on STN: 20000303
Entered Medline: 19971212
AB Prion diseases are characterized by the conversion of the normal cellular prion protein (PrPC), a glycoprotein that is anchored to the cell membrane
by a glycosylphosphatidylinositol moiety, into an isoform that is protease-resistant (PrPres) and pathogenic. In inherited prion diseases, mutations in the prion protein (PrPM) engender the conversion of PrPM into
PrPres. We developed a cell model of Gerstmann-Straussler-Scheinker disease, a neurodegenerative condition characterized by PrPM-containing **amyloid** deposits and neuronal loss, by expressing the Gerstmann-Straussler-Scheinker haplotype Q217R-129V in human neuroblastoma
cells. By comparison to PrPC, this genotype results in the following alterations of PrPM: 1) expression of an aberrant form lacking the glycosylphosphatidylinositol anchor, 2) increased **aggregation** and **protease resistance**, and 3) impaired transport to the cell surface. Most of these alterations are temperature-sensitive, indicating that they are due to misfolding of PrPM.

L2 ANSWER 6 OF 10 MEDLINE
AN 97338067 MEDLINE
DN 97338067 PubMed ID: 9192614
TI Prion-inducing domain 2-114 of yeast Sup35 protein transforms in vitro into **amyloid**-like filaments.
AU King C Y; Tittmann P; Gross H; Gebert R; Aebi M; Wuthrich K
CS Institut fur Molekularbiologie und Biophysik, Eidgenossische Technische Hochschule, CH-8093 Zurich, Switzerland.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Jun 24) 94 (13) 6618-22.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199707
ED Entered STN: 19970805
Last Updated on STN: 19970805
Entered Medline: 19970721
AB The yeast non-Mendelian genetic factor [PSI], which enhances the efficiency of tRNA-mediated nonsense suppression in Saccharomyces

cerevisiae, is thought to be an abnormal cellular isoform of the Sup35 protein. Genetic studies have established that the N-terminal part of the Sup35 protein is sufficient for the genesis as well as the maintenance of [PSI]. Here we demonstrate that the N-terminal polypeptide fragment consisting of residues 2-114 of Sup35p, Sup35pN, spontaneously **aggregates** to form thin filaments in vitro. The filaments show a beta-sheet-type circular dichroism spectrum, exhibit increased **protease resistance**, and show **amyloid**-like optical properties. It is further shown that filament growth in freshly prepared Sup35pN solutions can be induced by seeding with a dilute suspension of preformed filaments. These results suggest that the abnormal cellular isoform of Sup35p is an **amyloid**-like aggregate and further indicate that seeding might be responsible for the maintenance of the [PSI] element in vivo.

L2 ANSWER 7 OF 10 MEDLINE
AN 97293259 MEDLINE
DN 97293259 PubMed ID: 9148807
TI Effectiveness of anthracycline against experimental prion disease in Syrian hamsters.
AU Tagliavini F; McArthur R A; Canciani B; Giaccone G; Porro M; Bugiani M; Lievens P M; Bugiani O; Peri E; Dall'Ara P; Rocchi M; Poli G; Forloni G; Bandiera T; Varasi M; Suarato A; Cassutti P; Cervini M A; Lansen J; Salmona M; Post C
CS Istituto Nazionale Neurologico Carlo Besta, via Celoria 11, 20133 Milano, Italy.
SO SCIENCE, (1997 May 16) 276 (5315) 1119-22.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199706
ED Entered STN: 19970612
Last Updated on STN: 19970612
Entered Medline: 19970603
AB Prion diseases are transmissible neurodegenerative conditions characterized by the accumulation of **protease-resistant** forms of the prion protein (PrP), termed PrPres, in the brain. Insoluble PrPres tends to **aggregate** into **amyloid** fibrils. The anthracycline 4'-iodo-4'-deoxy-doxorubicin (IDX) binds to **amyloid** fibrils and induces **amyloid** resorption in patients with systemic **amyloidosis**. To test IDX in an experimental model of prion disease, Syrian hamsters were inoculated intracerebrally either with scrapie-infected brain homogenate or with infected homogenate coincubated with IDX. In IDX-treated hamsters, clinical signs of disease were delayed and survival time was prolonged. Neuropathological examination showed a parallel delay in the appearance of brain changes and in the accumulation of PrPres and PrP **amyloid**.

L2 ANSWER 8 OF 10 MEDLINE
AN 93356816 MEDLINE
DN 93356816 PubMed ID: 8102526
TI Molecular characteristics of a protease-resistant, **amyloidogenic** and neurotoxic peptide homologous to residues 106-126 of the prion protein.
AU Selvaggini C; De Gioia L; Cantu L; Ghibaudi E; Diomede L; Passerini F; Forloni G; Bugiani O; Tagliavini F; Salmona M
CS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.

SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1993 Aug 16) 194 (3) 1380-6.
Journal code: 9Y8; 0372516. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199309
ED Entered STN: 19931001
Last Updated on STN: 20000303
Entered Medline: 19930915
AB In the prion-related encephalopathies the prion protein is converted to an altered form, known as PrPSc, that is partially **resistant** to **protease** digestion. This abnormal isoform accumulates in the brain and its **protease-resistant** core **aggregates** extracellularly into **amyloid** fibrils. We have investigated the conformational properties, **aggregation** behaviour and sensitivity to **protease** digestion of a synthetic peptide homologous to residues 106-126 of human PrP, which was previously found to form **amyloid**-like fibrils in vitro and displayed neurotoxic activity toward primary cultures of rat hippocampal neurons. A scrambled sequence of peptide PrP 106-126 was used as a control. By circular dichroism, PrP 106-126 exhibited a secondary structure composed largely of beta-sheet, whereas the scrambled sequence of PrP 106-126 showed a random coil structure. The beta-sheet content of PrP 106-126 was much higher in 200 mM phosphate buffer at pH 5.0 than in the same buffer at pH 7.0. Laser light scattering analysis showed that PrP 106-126 aggregated immediately after dissolution in 20 mM or 200 mM phosphate buffer, pH 5.0 and 7.0, whereas scrambled PrP 106-126 did not. PrP 106-126 aggregates had an average hydrodinamic diameter of 100 nm and an average molecular weight of 12 x 10(6) +/- 30% Daltons, corresponding to the aggregation of 6000 +/- 30% molecules. Peptide PrP 106-126 showed partial resistance to digestion with Proteinase K and Pronase, whereas scrambled PrP 106-126 was completely degraded by incubation with the enzymes at 37 degrees C for 30 minutes.

L2 ANSWER 9 OF 10 MEDLINE
AN 93218742 MEDLINE
DN 93218742 PubMed ID: 8464494
TI Neurotoxicity of a prion protein fragment.
AU Forloni G; Angeretti N; Chiesa R; Monzani E; Salmona M; Bugiani O; Tagliavini F
CS Istituto di Ricerche Farmacologiche Mario Negri, Milano, Italy.
SO NATURE, (1993 Apr 8) 362 (6420) 543-6.
Journal code: NSC; 0410462. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199305
ED Entered STN: 19930521
Last Updated on STN: 19930521
Entered Medline: 19930503
AB The cellular prion protein (PrPC) is a sialoglycoprotein of M(r) 33-35K that is expressed predominantly in neurons. In transmissible and genetic neurodegenerative disorders such as scrapie of sheep, spongiform encephalopathy of cattle and Creutzfeldt-Jakob or Gerstmann-Straussler-

Scheinker diseases of humans, PrPC is converted into an altered form (termed PrPSc) which is distinguishable from its normal homologue by its relative **resistance** to **protease** digestion. PrPSc accumulates in the central nervous system of affected individuals, and its **protease-resistant** core **aggregates** extracellularly into **amyloid** fibrils. The process is accompanied by nerve cell loss, whose pathogenesis and molecular basis are not understood. We report here that neuronal death results from chronic exposure of primary rat hippocampal cultures to micromolar concentrations of a peptide corresponding to residues 106-126 of the amino-acid sequence deduced from human PrP complementary DNA. DNA fragmentation of degenerating neurons indicates that cell death occurred by apoptosis. The PrP peptide 106-126 has a high intrinsic ability to polymerize into **amyloid**-like fibrils in vitro. These findings indicate that cerebral accumulation of PrPSc and its degradation products may play a role in the nerve cell degeneration that occurs in prion-related encephalopathies.

L2 ANSWER 10 OF 10 MEDLINE
AN 93167698 MEDLINE
DN 93167698 PubMed ID: 1288372
TI The lysosomal system in neurons. Involvement at multiple stages of Alzheimer's disease pathogenesis.
AU Nixon R A; Cataldo A M; Paskevich P A; Hamilton D J; Wheelock T R; Kanaley-Andrews L
CS Laboratory for Molecular Neuroscience, Mailman Research Center, McLean Hospital, Harvard Medical School, Belmont, Massachusetts 02178.
NC AG05134 (NIA)
AG08278 (NIA)
RO1-MH/NS31862 (NIMH)
SO ANNALS OF THE NEW YORK ACADEMY OF SCIENCES, (1992 Dec 31) 674 65-88.
Journal code: 5NM; 7506858. ISSN: 0077-8923.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199303
ED Entered STN: 19930402
Last Updated on STN: 19980206
Entered Medline: 19930315
AB Disturbed lysosomal function may be implicated at several stages of Alzheimer's pathogenesis. Lysosomes and acid hydrolases accumulate in the majority of neocortical pyramidal neurons before typical degenerative changes can be detected, indicating that altered lysosome function is among the earliest markers of metabolic dysfunction in Alzheimer's disease. These early alterations could reflect accelerated membrane and protein turnover, defective lysosome or hydrolase function, abnormal lysosomal trafficking or any combination of these possibilities. Because APP is partly metabolized in lysosomes, early disturbances in lysosomal function could promote the production of abnormal and/or neurotoxic APP fragments within intact cells. Lysosomal abnormalities progressively worsen as neurons begin to degenerate. Based on existing literature on cell death, increased perturbation and instability of the lysosomal system may be expected to contribute to the atrophy and eventual lysis of the neuron. Finally, the release of hydrolase-filled lysosomes and lipofuscin aggregates from dying neurons accounts for the abundant deposition of enzymatically active acid hydrolases of all classes in the extracellular space--a phenomenon that may be unique to Alzheimer's disease. Acting on

APP present in surrounding dystrophic neurites, cellular debris and astrocyte processes, dysregulated hydrolases may cleave APP in atypical sequential patterns, thereby generating self-**aggregating** **protease-resistant** APP fragments that can be only processed to beta-**amyloid**. Genetic mutations or posttranslational factors of APP should further enhance the generation of **amyloidogenic** fragments by a dysregulated lysosomal system. Given that very little, if any, beta-**amyloid** is detected intracellularly, yet extracellular beta-**amyloid** is very abundant, our data suggest that the final steps of APP processing and the generation of most beta-**amyloid** in the brain parenchyma occur extracellularly and may involve one or more lysosomal proteases.

=>

A .beta.A4-C-terminal construct accumulated into membranous structures in the cytoplasm and nucleus and reacted with most antibodies to .beta.A4 and the cytoplasmic domain of A.beta.PP. The two shorter constructs contg. the .beta.A4 sequence formed similar intranuclear **aggregates** to those reported for intranuclear inclusions of polyglutamine peptides from huntingtin (in Huntington's disease) and ataxin protein fragments (in spinocerebellar ataxia). This is of interest because intracellular **aggregation** of the polyglutamine and .beta.A4 peptides may affect cells by similar toxic mechanisms. These studies demonstrate clear differences in the expression properties of different A.beta.PP polypeptides.

L14 ANSWER 11 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:377065 CAPLUS
DN 129:119235
TI The surveillance complex interacts with the translation release factors to enhance termination and degrade aberrant mRNAs
AU Czaplinski, Kevin; Ruiz-Echevarria, Maria J.; Paushkin, Sergey V.; Han, Xia; Weng, Youmin; Perlick, Haley A.; Dietz, Harry C.; Ter-Avanesyan, Michael D.; Peltz, Stuart W.
CS Department of Molecular Genetics and Microbiology, University of Medicine and Dentistry of New Jersey (UMDNJ)/Rutgers Universities, Piscataway, NJ, 08854, USA
SO Genes Dev. (1998), 12(11), 1665-1677
CODEN: GEDEEP; ISSN: 0890-9369
PB Cold Spring Harbor Laboratory Press
DT Journal
LA English
AB The nonsense-mediated mRNA decay pathway is an example of an evolutionarily conserved surveillance pathway that rids the cell of transcripts that contain nonsense mutations. The product of the UPF1 gene is a necessary component of the putative surveillance complex that recognizes and degrades aberrant mRNAs. Recent results indicate that the Upf1p also enhances translation termination at a nonsense codon. The results presented here demonstrate that the **yeast** and human forms of the Upf1p interact with both eukaryotic translation termination factors eRF1 and eRF3. Consistent with Upf1p interacting with the eRFs, the Upf1p is found in the **prion**-like **aggregates** that contain eRF1 and eRF3 obsd. in **yeast** [PSI+] strains. These results suggest that interaction of the Upf1p with the peptidyl release factors may be a key event in the assembly of the putative surveillance complex that enhances translation termination, monitors whether termination has occurred prematurely, and promotes degrdn. of aberrant transcripts.

L14 ANSWER 12 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:209231 CAPLUS
DN 128:320134
TI .alpha.2-macroglobulin associates with .beta.-**amyloid** peptide and prevents fibril formation
AU Hughes, Stephen R.; Khorkova, Olga; Goyal, Shefali; Knaeblein, Joerg; Heroux, Jeffrey; Riedel, Norbert G.; Sahasrabudhe, Sudhir
CS Biotechnol. Group and the Central Nervous System Disease Group, Hoechst Marion Roussel, Inc., Bridgewater, NJ, 08876-0800, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1998), 95(6), 3275-3280
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB We have used the **yeast** two-hybrid system to isolate cDNAs encoding proteins that specifically interact with the 42-aa .beta.-**amyloid** peptide (A.beta.), a major constituent of senile **plaques** in Alzheimer's disease. The carboxy terminus of

L3 ANSWER 1 OF 1 MEDLINE
AN 96279350 MEDLINE
DN 96279350 PubMed ID: 8663372
TI The serpin-enzyme complex receptor recognizes soluble, nontoxic amyloid-beta peptide but not aggregated, cytotoxic amyloid-beta peptide.
AU Boland K; Behrens M; Choi D; Manias K; Perlmutter D H
CS Department of Pediatrics, Washington University School of Medicine, St. Louis, Missouri 63110, USA.
NC AG11577 (NIA)
HL-37784 (NHLBI)
NS30337 (NINDS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Jul 26) 271 (30) 18032-44.
Journal code: HIV; 2985121R. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199609
ED Entered STN: 19960912
Last Updated on STN: 19970203
Entered Medline: 19960903
AB There is now extensive evidence that amyloid-beta peptide is toxic to neurons and that its cytotoxic effects can be attributed to a domain corresponding to amyloid-beta 25-35, GSNKGAIIGLM. We have shown recently that the serine proteinase inhibitor (serpin)-enzyme complex receptor (SEC-R), a receptor initially identified for binding of alpha1-antitrypsin (alpha1-AT) and other serine protease inhibitors, also recognizes the amyloid-beta 25-35 domain. In fact, by recognizing the amyloid-beta 25-35 domain, SEC-R mediates cell surface binding, internalization, and degradation of soluble amyloid-beta peptide. In this study, we examined the possibility that SEC-R mediates the neurotoxic effect of amyloid-beta peptide. A series of peptides based on the sequences of amyloid-beta peptide and alpha1-AT was prepared soluble in dimethyl sulfoxide or insoluble in water and examined in assays for SEC-R binding, for cytotoxicity in neuronal PC12 cells and murine cortical neurons in primary culture, and for aggregation in sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analysis. The results show that amyloid-beta peptide 25-35 and amyloid-beta peptide 1-40 prepared soluble in dimethyl sulfoxide compete for binding to SEC-R, are nontoxic, and migrate as monomers in SDS-PAGE analysis. In contrast, the same peptides aged in water did not compete for binding to SEC-R but were toxic and migrated as aggregates in SDS-PAGE. An all-D-**amyloid**-beta 25-35 peptide was not recognized at all by SEC-R but retained full toxic/**aggregating** properties. Using a series of deleted, substituted, and **chimeric** ambeta/alpha1-AT peptides, toxicity correlated well with aggregation but poorly with SEC-R recognition. In a subclone of PC12 cells which developed resistance to the toxic effect of aggregated amyloid-beta 25-35 there was a 2.5-3-fold increase in the number of SEC-R molecules/cell compared with the parent PC12 cell line. These data show that SEC-R does not mediate the cytotoxic effect of aggregated amyloid-beta peptide. Rather, SEC-R could play a protective role by mediating clearance and catabolism of soluble, monomeric amyloid-beta peptide, if soluble amyloid-beta peptide proves to be an in vivo precursor of the insoluble, toxic peptide.

1022

L6 ANSWER 1 OF 28 CAPLUS COPYRIGHT 1999 ACS
AN 1999:113797 CAPLUS
DN 130:166800
TI Soluble fusion proteins of aggregate-forming proteins and the study of diseases associated with protein aggregate formation
IN Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian
PA Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany
SO PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9906545	A2	19990211	WO 98-EP4811	19980731

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

AB Fusion proteins of aggregate-forming proteins and solubilizing peptides are described for use in elucidating the mechanism, onset or progress of diseases assocd. with the formation of amyloid-like fibrils or protein aggregates. The method is for use in the study of neurol. diseases such as Huntington's and Alzheimer's. The fusion proteins can also be used to screen for **inhibitors** of **aggregation** that may be of therapeutic use. Genes for a series of fusion proteins polyglutamine repeat expansion variants (20, 30, or 51 glutamine repeats) of huntingtin and glutathione-S-transferase were constructed by std. methods and manufd. in Escherichia coli using a hexahistidine for affinity purifn. The fusion proteins were sol. but cleavage of the 51 glutamine repeat variant (HD51) with trypsin led to the formation of insol. aggregates of the huntingtin. HD51 aggregated in vitro to form amyloid-like birefringent fibrils after liberation by trypsin cleavage, but the shorter repeat variants HD20 and HD30 did not do so. Similar effects were seen in vivo in COS-1 cells.

L6 ANSWER 2 OF 28 CAPLUS COPYRIGHT 1999 ACS
AN 1998:688033 CAPLUS
DN 130:35563
TI Mechanism of inhibition of .PSI.+ prion determinant propagation by a mutation of the N-terminus of the yeast Sup35 protein
AU Kochneva-Pervukhova, Natalia V.; Paushkin, Sergey V.; Kushnirov, Vitaly V.; Cox, Brian S.; Tuite, Mick F.; Ter-Avanesyan, Michael D.
CS Cardiology Research Center, Institute of Experimental Cardiology, Moscow, 121552, Russia
SO EMBO J. (1998), 17(19), 5805-5810
CODEN: EMJODG; ISSN: 0261-4189
PB Oxford University Press
DT Journal
LA English
AB The SUP35 gene of **Saccharomyces cerevisiae** encodes the polypeptide chain release factor eRF3. This protein (also called Sup35p) is thought to be able to undergo a heritable conformational switch, similarly to mammalian prions, giving rise to the cytoplasmically inherited .PSI.+ determinant. A dominant mutation (PNM2 allele) in the SUP35 gene causing a Gly58.fwdarw.Asp change in the Sup35p N-terminal domain eliminates .PSI.+. Here we obsd. that the mutant Sup35p can be converted to the prion-like form in vitro, but such conversion proceeds slower than that of wild-type Sup35p. The overexpression of mutant Sup35p

induced the de novo appearance of .PSI.+ cells contg. the prion-like form of mutant Sup35p, which was able to transmit its properties to wild-type Sup35p both in vitro and in vivo. Our data indicate that this .PSI.+-eliminating mutation does not alter the initial binding of Sup35p mols. to the Sup35p .PSI.+-specific **aggregates**, but rather **inhibits** its subsequent prion-like rearrangement and/or binding of the next Sup35p mol. to the growing prion-like Sup35p aggregate.

L6 ANSWER 4 OF 28 CAPLUS COPYRIGHT 1999 ACS
AN 1997:275947 CAPLUS
DN 126:327804
TI Interaction between yeast Sup45p (eRF1) and Sup35p (eRF3) polypeptide chain release factors: implications for prion-dependent regulation
AU Paushkin, Sergey V.; Kushnirov, Vitaly V.; Smirnov, Vladimir N.; Ter-Avanesyan, Michael D.
CS Inst. Exp. Cardiol., Cardiol. Res. Cent., Moscow, 121552, Russia
SO Mol. Cell. Biol. (1997), 17(5), 2798-2805
CODEN: MCEBD4; ISSN: 0270-7306
PB American Society for Microbiology
DT Journal
LA English
AB The SUP45 and SUP35 genes of **Saccharomyces cerevisiae** encode polypeptide chain release factors eRF1 and eRF3, resp. It has been suggested that the Sup35 protein (Sup35p) is subject to a heritable conformational switch, similar to mammalian prions, thus giving rise to the non-Mendelian [PSI+] nonsense suppressor determinant. In a [PSI+] state, Sup35p forms high-mol.-wt. **aggregates** which may **inhibit** Sup35p activity, leading to the [PSI+] phenotype. Sup35p is composed of the N-terminal domain (N) required for [PSI+] maintenance, the presumably nonfunctional middle region (M), and the C-terminal domain (C) essential for translation termination. In this study, we obsd. that the N domain, alone or as a part of larger fragments, can form aggregates in [PSI+] cells. Two sites for Sup45p binding were found within Sup35p: one is formed by the N and M domains, and the other is located within the C domain. Similarly to Sup35p, in [PSI+] cells Sup45p was found in aggregates. The aggregation of Sup45p is caused by its binding to Sup35p and was not obsd. when the aggregated Sup35p fragments did not contain sites for Sup45p binding. The incorporation of Sup45p into the **aggregates** should **inhibit** its activity. The N domain of Sup35p, responsible for its aggregation in [PSI+] cells, may thus act as a repressor of another polypeptide chain release factor, Sup45p. This phenomenon represents a novel mechanism of regulation of gene expression at the posttranslational level.

L6 ANSWER 5 OF 28 CAPLUS COPYRIGHT 1999 ACS
AN 1996:394350 CAPLUS
DN 125:53283
TI Propagation of the yeast prion-like [psi+] determinant is mediated by oligomerization of the SUP35-encoded polypeptide chain release factor
AU Kushniin, Sergey V.; Kushnirov, Vitaly V.; Smirnov, Vladimir N.; Ter-Avanesyan, Michael D.
CS Cardiology Res. Center, Inst. Experimental Cardiology, Moscow, 121552, Russia
SO EMBO J. (1996), 15(12), 3127-3134
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB The Sup35p protein of yeast **Saccharomyces cerevisiae** is a homolog of the polypeptide chain release factor 3 (eRF3) of higher eukaryotes. It has been suggested that this protein may adopt a specific self-propagating conformation, similar to mammalian prions, giving rise to the [psi+] nonsense suppressor determinant, inherited in a non-Mendelian fashion. Here, the authors present data confirming the prion-like nature

of [psi+]. They show that Sup35p mols. interact with each other through their N-terminal domains in [psi+], but not [psi-], cells. This interaction is crit. for [psi+] propagation, since its disruption leads to a loss of [psi+]. Similarly to mammalian prions, in [psi+] cells Sup35p forms high mol. wt. aggregates, accumulating most of this protein. The **aggregation inhibits** Sup35p activity, leading to a [psi+] nonsense-suppressor phenotype. N-terminally altered Sup35p mols. are unable to interact with the [psi+] Sup35p isoform, remain sol. and improve the translation termination in [psi+] strains, thus causing an antisuppressor phenotype. The overexpression of Hsp104p chaperone protein partially solubilizes Sup35p aggregates in the [psi+] strain, also causing an antisuppressor phenotype. The authors propose that Hsp104p plays a role in establishing stable [psi+] inheritance by splitting up Sup35p aggregates and thus ensuring equidistribution of the prion-like Sup35p isoform to daughter cells at cell divisions.

L6 ANSWER 6 OF 28 CAPLUS COPYRIGHT 1999 ACS
AN 1996:296803 CAPLUS
DN 125:28746
TI Characterization of an acid trehalase of **Saccharomyces cerevisiae** present in trehalase-sucrase aggregate
AU Biswas, Nilima; Ghosh, Anil Kumar
CS Applied Biochemistry Department, Indian Institute of Chemical Biology, 4, Raja S.C. Mullick Road, Calcutta, 700 032, India
SO Biochim. Biophys. Acta (1996), 1290(1), 95-100
CODEN: BBACAQ; ISSN: 0006-3002
DT Journal
LA English
AB An acid trehalase-sucrase aggregate was purified (by 780-fold) from **Saccharomyces cerevisiae**, following conventional protein purifn. techniques, to an apparent yield of 18.5. The aggregate was electrophoretically homogeneous but contained 175, 90, 68, 60, 40 M mass (kDa) bands on SDS-electrophoresis. The purified aggregate had a specific activity (acid trehalase) of 22 U/mg; a Km value of 5.0 mM but contained 3-times more sucrase activity. Only sucrose and trehalose were hydrolyzed by this **aggregate**, and both activities were **inhibited** by acetate or phosphate. Temp. and pH optima for trehalose hydrolysis appeared to be 40-45.degree. and 5.0, resp. The purified aggregate appeared to be disaggregating spontaneously resulting in inactivation of both enzymes, which was enhanced either at pH 3.5 or at pH 7.0. Sepn. of acid trehalase from the aggregate by hydrophobic interaction chromatog. resulted in inactivation. Rechromatog. (HPGPLC) of the purified aggregate also gave disaggregation as well as inactivation of both enzymes. Disaggregated acid trehalase and sucrase contained 20-fold and 13-fold lower specific activities, resp., and appeared to be unstable. Based on these observations the authors suggest that acid trehalase is stabilized by aggregation with sucrase.

DN 115:273625
TI Reconstitution of a heat shock effect in vitro: influence of GroE on the thermal aggregation of .alpha.-glucosidase from yeast
AU Hoell-Neugebauer, Baerbel; Rudolph, Rainer; Schmidt, Marion; Buchner, Johannes
CS Biochem. Res. Cent., Boehringer Mannheim G.m.b.H., Penzberg, D-8122, Fed. Rep. Ger.
SO Biochemistry (1991), 30(50), 11609-14
CODEN: BICHAW; ISSN: 0006-2960
DT Journal
LA English
AB .alpha.-Glucosidase from yeast is inactivated rapidly at temps. above 42.degree.. The thermal inactivation is accompanied by aggregation. The mol. chaperone GroEL suppresses the formation of aggregates by binding the thermally inactivated .alpha.-glucosidase. Spectroscopic studies suggest that GroEL binds .alpha.-glucosidase in an intermediately folded state. The complex between .alpha.-glucosidase and GroEL can be dissolved by MgATP. GroES accelerates the MgATP-dependent dissocn. of the .alpha.-glucosidase-GroEL complex. At elevated temps. this release leads to the formation of aggregates, while at lower temps. native, enzymically active

L3 ANSWER 15 OF 17 MEDLINE
AN 1998054338 MEDLINE
DN 98054338 PubMed ID: 9391130
TI Interactions of the chaperone Hsp104 with yeast **Sup35** and mammalian PrP.
AU Schirmer E C; Lindquist S
CS Department of Molecular Genetics and Cell Biology and Howard Hughes Medical Institute, University of Chicago, Chicago, IL, 60637, USA.
NC GM25874 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1997 Dec 9) 94 (25) 13932-7.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199801
ED Entered STN: 19980129
Last Updated on STN: 19980129
Entered Medline: 19980115
AB [PSI+] is a genetic element in yeast for which a heritable change in phenotype appears to be caused by a heritable change in the conformational state of the **Sup35** protein. The inheritance of [PSI+] and the physical state of **Sup35** in vivo depend on the protein chaperone Hsp104 (heat shock protein 104). Although these observations provide a strong genetic argument in support of the "protein-only" or "prion" hypothesis for [PSI+], there is, as yet, no direct evidence of an interaction between the two proteins. We report that when purified **Sup35** and Hsp104 are mixed, the circular dichroism (CD) spectrum differs from that predicted by the addition of the proteins' individual spectra, and the ATPase activity of Hsp104 is inhibited. Similar results are obtained with two other **amyloidogenic** substrates, mammalian PrP and beta-**amyloid** 1-42 peptide, but not with several control proteins. With a group of peptides that span the PrP protein sequence, those that produced the largest changes in CD spectra also caused the strongest inhibition of ATPase activity in Hsp104. Our observations suggest that (i) previously described genetic interactions between Hsp104 and [PSI+] are caused by direct interaction between Hsp104 and **Sup35**; (ii) **Sup35** and PrP, the determinants of the yeast and mammalian prions, respectively, share structural features that lead to a specific interaction with Hsp104; and (iii) these interactions couple a change in structure to the ATPase activity of Hsp104.

L5 ANSWER 17 OF 18 MEDLINE
AN 97476303 MEDLINE
DN 97476303 PubMed ID: 9335589
TI Genetic and environmental factors affecting the de novo appearance of the [PSI+] prion in Saccharomyces cerevisiae.
AU Derkatch I L; Bradley M E; Zhou P; Chernoff Y O; Liebman S W
CS Department of Biological Sciences, University of Illinois at Chicago 60607, USA.
NC 1R21GM-55091-01 (NIGMS)
1RO1GM-56350-01 (NIGMS)
SO GENETICS, (1997 Oct) 147 (2) 507-19.
Journal code: FNH; 0374636. ISSN: 0016-6731.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199712
ED Entered STN: 19980109
Last Updated on STN: 19980109
Entered Medline: 19971208
AB It has previously been shown that yeast prion [PSI+] is cured by GuHCl, although reports on reversibility of curing were contradictory. Here we show that GuHCl treatment of both [PSI+] and [psi-] yeast strains results in two classes of [psi-] derivatives: Pin+, in which [PSI+] can be reinduced by Sup35p overproduction, and Pin-, in which **overexpression** of the complete SUP35 gene does not lead to the [PSI+] appearance. However, in both Pin+ and Pin- derivatives [PSI+] is reinduced by overproduction of a short Sup35p N-terminal fragment, thus, in principle, [PSI+] curing remains reversible in both cases. Neither suppression nor growth inhibition caused by SUP35 **overexpression** in Pin+ [psi-] derivatives are observed in Pin- [psi-] derivatives. Genetic analyses show that the Pin+ phenotype is determined by a non-Mendelian factor, which, unlike the [PSI+] prion, is independent of the Sup35p N-terminal domain. A Pin- [psi-] derivative was also generated by transient inactivation of the heat shock protein, **Hsp104**, while [PSI+] curing by **Hsp104** overproduction resulted exclusively in Pin+ [psi-] derivatives. We hypothesize that in addition to the [PSI+] prion-determining domain in the Sup35p N-terminus, there is another self-propagating conformational determinant in the C-proximal part of Sup35p and that this second prion is responsible for the Pin+ phenotype.

L9 ANSWER 5 OF 6 MEDLINE
AN 2000214816 MEDLINE
DN 20214816 PubMed ID: 10749925
TI Axonal membrane proteins are transported in distinct carriers: a two-color
video microscopy study in cultured hippocampal neurons.
AU Kaether C; Skehel P; Dotti C G
CS European Molecular Biology Laboratory, Cell Biology Program, 69012 Heidelberg, Germany.
SO MOLECULAR BIOLOGY OF THE CELL, (2000 Apr) 11 (4) 1213-24.
Journal code: BAU; 9201390. ISSN: 1059-1524.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200007
ED Entered STN: 20000720
Last Updated on STN: 20000720
Entered Medline: 20000711
AB Neurons transport newly synthesized membrane proteins along axons by microtubule-mediated fast axonal transport. Membrane proteins destined for
different axonal subdomains are thought to be transported in different transport carriers. To analyze this differential transport in living neurons, we tagged the **amyloid** precursor protein (APP) and synaptophysin (p38) with **green fluorescent protein** (GFP) variants. The resulting **fusion** proteins, APP-yellow fluorescent protein (YFP), p38-enhanced GFP, and p38-enhanced cyan fluorescent protein, were expressed in hippocampal neurons, and the cells were imaged by video microscopy. APP-YFP was transported in elongated tubules that moved extremely fast (on average 4.5 micrometer/s) and over long distances. In contrast, p38-enhanced GFP-transporting structures were more vesicular and moved four times slower (0.9 micrometer/s) and over shorter distances only. Two-color video microscopy showed that the two proteins were sorted to different carriers that moved with different characteristics along axons of doubly transfected neurons. Antisense treatment using oligonucleotides against the kinesin heavy chain
slowed down the long, continuous movement of APP-YFP tubules and increased
frequency of directional changes. These results demonstrate for the first time directly the sorting and transport of two axonal membrane proteins into different carriers. Moreover, the extremely fast-moving tubules represent a previously unidentified type of axonal carrier.

L9 ANSWER 4 OF 6 MEDLINE
AN 2001127829 MEDLINE
DN 20572085 PubMed ID: 11123686
TI The relationship between visible intracellular aggregates that appear after overexpression of **Sup35** and the yeast prion-like elements [PSI(+)] and [PIN(+)].
AU Zhou P; Derkatch I L; Liebman S W
CS Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL 60607, USA.
NC GM56350 (NIGMS)
SO MOLECULAR MICROBIOLOGY, (2001 Jan) 39 (1) 37-46.
Journal code: MOM. ISSN: 0950-382X.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 200102
ED Entered STN: 20010322
Last Updated on STN: 20010322
Entered Medline: 20010222
AB Overproduced **fusions** of **Sup35** or its prion domain with **green fluorescent protein** (GFP) have previously been shown to form frequent dots in [PSI(+)] cells. Rare foci seen in [psi(-)] cells were hypothesized to indicate the de novo induction of [PSI(+)] caused by the overproduced prion domain. Here, we describe novel ring-type aggregates that also appear in [psi(-)] cultures upon **Sup35** overproduction and show directly that dot and ring aggregates only appear in cells that have become [PSI(+)]. The formation of either type of aggregate requires [PIN(+)], an element needed for the induction of [PSI(+)]. Although aggregates are visible predominantly in stationary-phase cultures, [PSI(+)] induction starts in exponential phase, suggesting that much smaller aggregates can also propagate [PSI(+)]. Such small aggregates are probably present in [PSI(+)] cells and, upon **Sup35**-GFP overproduction, facilitate the frequent formation of dot aggregates, but only the occasional appearance of ring aggregates. In contrast, rings are very frequent when [PSI(+)] cultures, including those lacking [PIN(+)], are grown in the presence of GuHCl or excess Hsp104 while overexpressing **Sup35**-GFP. Thus, intermediates formed during [PSI(+)] curing seem to facilitate ring formation. Surprisingly, GuHCl and excess Hsp104, which are known to promote loss of [PSI(+)], did not prevent the de novo induction of [PSI(+)] by excess **Sup35** in [psi(-)][PIN(+)] strains.

L9 ANSWER 6 OF 6 MEDLINE
AN 96325424 MEDLINE
DN 96325424 PubMed ID: 8662547
TI Support for the prion hypothesis for inheritance of a phenotypic trait in yeast.
CM Comment in: Science. 1996 Aug 2;273(5275):580
AU Patino M M; Liu J J; Glover J R; Lindquist S
CS Howard Hughes Medical Institute and the Department of Molecular Genetics and Cell Biology, University of Chicago, 5841 South Maryland Avenue, Chicago, IL 60637, USA.
NC GM25874 (NIGMS)
SO SCIENCE, (1996 Aug 2) 273 (5275) 622-6.
Journal code: UJ7; 0404511. ISSN: 0036-8075.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199609
ED Entered STN: 19960912
Last Updated on STN: 19980206
Entered Medline: 19960903
AB A cytoplasmically inherited genetic element in yeast, [PSI+], was confirmed to be a prionlike aggregate of the cellular protein **Sup35** by differential centrifugation analysis and microscopic localization of a **Sup35-green fluorescent protein fusion**. Aggregation depended on the intracellular concentration and functional state of the chaperone protein Hsp104 in the same manner as did [PSI+] inheritance. The amino-terminal and carboxy-terminal domains of **Sup35** contributed to the unusual behavior of [PSI+]. [PSI+] altered the conformational state of newly synthesized prion proteins, inducing them to aggregate as well, thus fulfilling a major tenet of the prion hypothesis.

L14 ANSWER 9 OF 10 USPATFULL
AN 97:106940 USPATFULL
TI Prevention of protein aggregation
IN Solomon, Beka, Herzlya, Israel
PA RAMOT University Authority For Applied Research and Development Ltd., Tel Aviv, Israel (non-U.S. corporation)
PI US 5688651 19971118
AI US 1994-358786 19941216 (8)
DT Utility
EXNAM Primary Examiner: Feisee, Lila; Assistant Examiner: Eyler, Yvonne
LREP Kohn & Associates
CLMN Number of Claims: 4
ECL Exemplary Claim: 1
DRWN 9 Drawing Figure(s); 3 Drawing Page(s)
LN.CNT 1212
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB A method of selecting anti-aggregation molecules with chaperone-like activity that have characteristics including binding to a native target molecule epitope with a high binding constant and are non-inhibitory to the biological activity of the target molecule. The method molecules denaturating a target molecule in the presence of presumptative antiaggregation molecules to prevent the target molecules from self-or induced-aggregation. The nonaggregated target molecule coupled to the anti

5221607

5854204

L3 ANSWER 14 OF 223 MEDLINE
AN 96050923 MEDLINE
DN 96050923
TI Insertion of a pathogenic mutation into a **yeast** artificial chromosome containing the human **amyloid** precursor protein gene.
AU Duff K; McGuigan A; Huxley C; Schulz F; Hardy J
CS Suncoast Alzheimer's Disease Laboratories, Department of Psychiatry, University of South Florida, Tampa 33613, USA.
NC RO1 AG11871-01 (NIA)
SO GENE THERAPY, **(1994 Jan)** 1 (1) 70-5.
Journal code: CCE. ISSN: 0969-7128.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199602
TI Insertion of a pathogenic mutation into a **yeast** artificial chromosome containing the human **amyloid** precursor protein gene.
SO GENE THERAPY, **(1994 Jan)** 1 (1) 70-5.
Journa

Trends in Microbiol. 1995 Oct 3(10):367-9
Review of yeast: hsp104 (copper)

DN 95050540
TI Proteolytic processing and secretion of human beta-**amyloid** precursor protein in **yeast**. Evidence for a **yeast** secretase activity.
AU Zhang H; Komano H; Fuller R S; Gandy S E; Frail D E
CS Department of Corporate Molecular Biology, Abbott Laboratories, Abbott Park, Illinois 60064.
NC GM39697 (NIGMS)
AG11508 (NIA)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 Nov 11)** 269 (45) 27799-802.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199502
TI Proteolytic processing and secretion of human beta-**amyloid** precursor protein in **yeast**. Evidence for a **yeast** secretase activity.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, **(1994 Nov 11)** 269 (45) 27799-802.
Journal code: HIV. ISSN: 0021-9258.
AB Human beta-**amyloid** precursor protein (APP); the transmembrane precursor of the Alzheimer's disease beta-**amyloid** peptide, was expressed in the **yeast** Saccharomyces cerevisiae by fusion to prepro-alpha-factor. From analysis of protease-deficient **yeast** strains, the fusion protein underwent partial processing by Kex2 protease to gen

L5 ANSWER 6 OF 12 MEDLINE
AN 95170764 MEDLINE
DN 95170764
TI The expression and processing of human beta-**amyloid** peptide precursors in Saccharomyces cerevisiae: evidence for a novel endopeptidase in the **yeast** secretory system.
AU Hines V; Zhang W; Ramakrishna N; Styles J; Mehta P; Kim K S; Innis M; Miller D L
CS Department of Microbial Expression, Chiron Corp., Emeryville, CA 94608.
NC AG 04220 (NIA)
SO CELLULAR AND MOLECULAR BIOLOGY RESEARCH, **(1994)** 40 (4) 273-84.
Journal code: BSK. ISSN: 0968-8773.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199506
TI The expression and processing of human beta-**amyloid** peptide precursors in Saccharomyces cerevisiae: evidence for a novel endopeptidase in the **yeast** secretory system.
SO CELLULAR AND MOLECULAR BIOLOGY RESEARCH, **(1994)** 40 (4) 273-84.
Journal code: BSK. ISSN: 0968-8773.
AB . . . is also reinternalized and degraded in the endosomal-lysosomal system. The relative efficiencies of these competing processes determine the yield of beta-**amyloid** peptide. Several proteases have been implicated in this complex processing pathway, although none has been identified to date. The **yeast** secretory system contains proteases homologous to mammalian pro-hormone convertases and is susceptible to genetic manipulation. We therefore investigated the expres

L5 ANSWER 4 OF 12 MEDLINE
AN 95367025 MEDLINE
DN 95367025
TI Expression, purification, and neurotrophic activity of **amyloid** precursor protein-secreted forms produced by **yeast**.
AU Ohsawa I; Hirose Y; Ishiguro M; Imai Y; Ishiura S; Kohsaka S
CS Department of Neurochemistry, National Institute of Neuroscience, Tokyo, Japan..
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, **(1995 Aug 4)** 213 (1) 52-8.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199511
TI Expression, purification, and neurotrophic activity of **amyloid** precursor protein-secreted forms produced by **yeast**.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, **(1995 Aug 4)** 213 (1) 52-8.
Journa

L11 ANSWER 14 OF 38 MEDLINE
AN 94139895 MEDLINE
DN 94139895
TI Expression, purification and characterization of a Kunitz-type protease inhibitor domain from human amyloid precursor protein homolog.
AU Petersen L C; Bjorn S E; Norris F; Norris K; Sprecher C; Foster D C
CS Novo Nordisk Research Institute, Gentofte, Denmark..
SO FEBS LETTERS, **(1994 Jan 24)** 338 (1) 53-7.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199405
SO FEBS LETTERS, **(1994 Jan 24)** 338 (1) 53-7.
Journal code: EUH. ISSN: 0014-5793.
AB The Kunitz-type protease inhibitor domain from a recently identified homolog of the Alzheimer **amyloid** precursor protein (APPH KPI) was **expressed** in **yeast**, purified and characterized. Its inhibition profile towards several serine proteases was studied and compared to that of APP KPI, the. . .

L11 ANSWER 6 OF 38 MEDLINE
AN 96224278 MEDLINE
DN 96224278
TI Heat-shock protein 104 expression is sufficient for thermotolerance in yeast.
AU Lindquist S; Kim G
CS Department of Molecular Genetics and Cell Biology, The University of Chicago, IL 60637, USA.
NC GM25874 (NIGMS)
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1996 May 28)** 93 (11) 5301-6.
Journal code: PV3. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199609
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, **(1996 May 28)** 93 (11) 5301-6.
Journal code: PV3. ISSN: 0027-8424.
AB . . . by heat, it did not block the induction of Hsp104. HSP104 could not be deleted in hsf1-m3 cells because the **expression** of heat-shock factor (and the viability of the strain) requires nonsense suppression mediated by the **yeast prion** [PSI+], which in turn depends upon Hsp104. To determine whether the level of Hsp104 **expressed** in hsf1-m3 cells is sufficient for thermotolerance, we used heterologous promoters to regulate Hsp104 expression in other strains. In the. . .

L11 ANSWER 16 OF 38 MEDLINE
AN 93384791 MEDLINE
DN 93384791
TI High level expression in Saccharomyces cerevisiae of an artificial gene encoding a repeated tripeptide aspartyl-phenylyalanyl-lysine.
AU Choi S Y; Lee S Y; Bock R M
CS Department of Agricultural Chemistry, Korea University, South Korea.
SO JOURNAL OF BIOTECHNOLOGY, **(1993 Aug)** 30 (2) 211-23.
Journal code: AL6. ISSN: 0168-1656.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS B
EM 199312
SO JOURNAL OF BIOTECHNOLOGY, **(1993 Aug)** 30 (2) 211-23.
Journal code: AL6. ISSN: 0168-1656.
AB . . . about 30% of the total cell protein. SDS-polyacrylamide gel electrophoresis and immunoblot analysis indicated that the artificial polypeptides synthesized in **yeast** have molecular weights ranging from about 30,000 and 60,000 and have immunoreactivity with the artificial
polypeptides **expressed** in E. coli. The artificial popypeptides in whole cell extract were insoluble and seem to be synthesized as insoluble **aggregates**. Electron microscopy showed the presence of inclusion bodies in the cell. These polypeptides can be hydrolyzed to tripeptides with trypsin. . .

L11 ANSWER 14 OF 38 MEDLINE
AN 94139895 MEDLINE
DN 94139895
TI Expression, purification and characterization of a Kunitz-type protease inhibitor domain from human amyloid precursor protein homolog.
AU Petersen L C; Bjorn S E; Norris F; Norris K; Sprecher C; Foster D C
CS Novo Nordisk Research Institute, Gentofte, Denmark..
SO FEBS LETTERS, **(1994 Jan 24)** 338 (1) 53-7.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199405
SO FEBS LETTERS, **(1994 Jan 24)** 338 (1) 53-7.
Journal code: EUH. ISSN: 0014-5793.
AB The Kunitz-type protease inhibitor domain from a recently identified homolog of the Alzheimer **amyloid** precursor protein (APPH KPI) was **expressed** in **yeast**, purified and characterized. Its inhibition profile towards several serine proteases was studied and compared to that of APP KPI, the. . .

AN 95:73612 USPATFULL
TI Human amyloid protein precursor homologue and Kunitz-type inhibitors
IN Sprecher, Cindy A., 8206 39th Ave. NE., Seattle, WA, United States 98115
Foster, Donald C., 3002 NE. 181st St., Seattle, WA, United States 98115
Norris, Kjeld E., Ahlmanns Alle 34, 2900 Hellerup, Denmark
PI US 5441931 19950815 <--
AI US 1993-155331 19931119 (8)
RLI Continuation-in-part of Ser. No. US 1992-985692, filed on 2 Dec 1992
DT Utility
EXNAM Primary Examiner: Patterson, Jr., Charles L.; Assistant Examiner: Kim, Hyosuk
LREP Sawislak, Deborah
CLMN Number of Claims: 3
ECL Exemplary Claim: 1
DRWN No Drawings
LN.CNT 1559
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
PI US 5441931 19950815 <--
DETD The Kunitz-type inhibitor domain of the **amyloid** protein precursor (SEQ ID NO:17) was **expressed** in a strain of the **yeast** Saccharomyces cerevisiae from a PCR-generated sequence.
The DNA sequence encoding the Kunitz-type inhibitor domain was amplified
from

L15 ANSWER 6 OF 19 MEDLINE
AN 95026291 MEDLINE
DN 95026291 PubMed ID: 7940017
TI Membrane-bound neomycin phosphotransferase confers drug-resistance in mammalian cells: a **marker** for high-efficiency targeting of genes encoding secreted and cell-surface proteins.
AU Mohler W A; Blau H M
CS Department of Molecular Pharmacology, Stanford University, California 94305-5332.
NC CA 59717 (NCI)
HD 07249-11 (NICHD)
HD 18179 (NICHD)
SO SOMATIC CELL AND MOLECULAR GENETICS, (1994 May) 20 (3) 153-62.
Journal code: UY2; 8403568. ISSN: 0740-7750.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199410
ED Entered STN: 19941222
Last Updated on STN: 19980206
Entered Medline: 19941027
AB An efficient method for inactivating genes is the use of silent selectable **markers** that are expressed only after homologous recombination into the active target gene. However, use of this approach for genes encoding secreted or membrane-anchored proteins may produce hybrid proteins comprising the N-terminal signal sequence from the target gene linked to the protein conferring **drug resistance**. Such **chimeric** enzymes will be secreted, precluding selection for **drug resistance**. To overcome this problem, we tested the possibility of anchoring in the membrane the cytoplasmic neomycin phosphotransferase (NPT). We constructed a fusion gene with a transmembrane domain connecting the N-terminal signal sequence of a membrane-targeted protein and the neo gene. Expression of this gene yielded G418-resistant colonies of C2C12 cells which contained assayable NPT activity. Comparison of enzyme activity in cell extract fractions verified that the active fusion protein was insoluble, presumably through localization to a membrane compartment. Transmembrane neo cassettes should serve as integration-activated **markers** capable of targeting genes encoding secreted or cell surface proteins.

103

L16 ANSWER 1 OF 2 MEDLINE
AN 94074545 MEDLINE
DN 94074545 PubMed ID: 8253072
TI **Chimeric** retinoic acid/thyroid **hormone receptors** implicate RAR-alpha 1 as mediating growth inhibition by retinoic acid.
AU Schilthuis J G; Gann A A; Brockes J P
CS Ludwig Institute for Cancer Research, University College London, UK.
SO EMBO JOURNAL, (1993 Sep) 12 (9) 3459-66.
Journal code: EMB; 8208664. ISSN: 0261-4189.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199401
ED Entered STN: 19940203
Last Updated on STN: 19940203
Entered Medline: 19940110
AB Retinoic acid (RA) affects the growth and differentiation of cells in culture, usually to decrease the growth rate. In amphibian limb regeneration RA has the remarkable ability to affect pattern formation by changing positional identity, but its initial action on the limb is to inhibit division of the blastemal progenitor cells. Newt limb blastemal cells also show this inhibition in culture. In order to investigate the role of different RA receptors (RARs) in the RA response, the hormone binding domain of the newt RARs alpha 1 and delta 1 was replaced with the corresponding region from the Xenopus thyroid **hormone receptor**-alpha (TR-alpha). In COS cells transfected with each of the **chimeras**, transcription was activated after exposure to thyroid hormone (T3). Their profile of activity on three different response elements was indicative of RAR specificity and not TR specificity. After transfection of cultured newt blastemal cells with a DNA particle gun, the chimeras were equally active in stimulating T3-dependent transcription of two different synthetic reporter genes. Blastemal cells were transfected with chimeras or control plasmids along with a **marker** plasmid expressing beta-galactosidase, exposed to RA or T3 and labelled with [3H]thymidine followed by autoradiography. The alpha 1 chimera gave T3-dependent inhibition of growth, comparable to the effect exerted by RA itself, whereas the delta 1 chimera and control plasmids were inactive. The results imply that RAR-alpha 1 mediates the effects of RA on blastemal cell growth.

L17 ANSWER 2 OF 3 MEDLINE
AN 96102222 MEDLINE
DN 96102222 PubMed ID: 8524871
TI Localization, trafficking, and temperature-dependence of the Aequorea green fluorescent protein in cultured vertebrate cells.
AU Ogawa H; Inouye S; Tsuji F I; Yasuda K; Umesono K
CS Graduate School of Biological Sciences, Nara Institute of Science and Technology, Japan.
SO PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA, (1995 Dec 5) 92 (25) 11899-903.
Journal code: PV3; 7505876. ISSN: 0027-8424.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199601
ED Entered STN: 19960219
Last Updated on STN: 19980206
Entered Medline: 19960124
AB The localization, trafficking, and fluorescence of Aequorea green fluorescent protein (GFP) in cultured vertebrate cells transiently transfected with GFP cDNA were studied. Fluorescence of GFP in UV light was found to be strongest when cells were incubated at 30 degrees C but was barely visible at an incubation temperature of 37 degrees C. COS-1 cells, primary chicken embryonic retina cells, and carp epithelial cells were fluorescently labeled under these conditions. GFP was distributed uniformly throughout the cytoplasm and nucleus independent of cell type examined. When GFP was fused to PML protooncogene product, fluorescence was detected in a unique nuclear organelle pattern indistinguishable from that of PML protein, showing the potential use of GFP as a fluorescent tag. To analyze both function and intracellular trafficking of proteins fused to GFP, a GFP-human **glucocorticoid receptor fusion** construct was prepared. The GFP-human **glucocorticoid receptor** efficiently transactivated the mouse mammary tumor virus promoter in response to dexamethasone at 30 degrees C but not at 37 degrees C, indicating that temperature is important, even for function of the GFP **fusion** protein. The dexamethasone-induced translocation of GFP-human **glucocorticoid receptor** from cytoplasm to nucleus was complete within 15 min; the translocation could be monitored in a single living cell in real time.

103

LA English
AB Several mammalian genes, including heat shock protein (Hsp70) and **prion** protein (**PrP**) genes, were reported to have long open reading frames (ORFs) or non-stop reading frames (NRFs) in the antisense direction. A simple explanation would be that these long antisense reading frames, which are usually in the same triplet frame as the coding strand, are the fortuitous byproduct of a high overall [G + C] content with concomitant preference for G/C over A/T in the 3rd codon position, a preference for RNY type codons (purine/any nucleotide/pyrimidine), and/or a bias against Ser and Leu, the only amino acids with codons that can be read as stop codons in the anti-sense direction. The **PrP** genes and most heat shock genes with long antisense NRFs (aNRFs) are indeed relatively [G + C] rich but do not show a bias against Ser and Leu. In several vertebrates investigated, at least one of the Hsp70 genes has a long anti-sense reading frame, and it was found that some, though not all, putative stop codons in long Hsp70 antisense reading frames were due to sequencing errors. The **PrP** gene contains an extended antisense open reading frame in all eutherian mammals tested, but not in a marsupial and in a bird. In the **PrP** gene, the long, protein-coding exon also harbors the antisense nonstop reading frame. In both Hsp70 and **PrP** genes, the putative antisense protein sequence is well conserved. Even though there is no clear evidence in Hsp70 or **PrP** genes for the existence of the resp. antisense proteins, it was speculated that such antisense proteins serve to regulate the genuine Hsp and **PrP** proteins under special circumstances. Alternatively, regulation might occur at the RNA level, and the anti-sense RNA would merely lack stop codons to prevent its rapid degrdn. by an mRNA quality control mechanism that is triggered by premature stop codons. Both Hsp and **PrP** are involved in physiol. or pathol. protein **aggregation** phenomena, that scrapie **prions** were reported to modify the expression or localization of heat shock proteins, and that in **yeast**, propagation of a **prion**-like state (PSI+) depends on a heat shock (Hsp104) protein.

L14 ANSWER 17 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1997:803246 CAPLUS
DN 128:137777
TI Interactions of the chaperone Hsp104 with **yeast Sup35** and mammalian **PrP**
AU Schirmer, Eric C.; Lindquist, Susan
CS Dep. Mol. Genetics Cell Biol., Howard Hughes Med. Inst., Univ. Chicago, Chicago, IL, 60637, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(25), 13932-13937
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB [PSI+] is a genetic element in **yeast** for which a heritable change in phenotype appears to be caused by a heritable change in the conformational state of the **Sup35** protein. The inheritance of [PSI+] and the phys. state of **Sup35** in vivo depend on the protein chaperone Hsp104 (heat shock protein 104). Although these observations provide a strong genetic argument in support of the "protein-only" or "**prion**" hypothesis for [PSI+], there is, as yet, no direct evidence of an interaction between the two proteins. We report that when purified **Sup35** and Hsp104 are mixed, the CD spectrum differs from that predicted by the addn. of the proteins' individual spectra, and the ATPase activity of Hsp104 is inhibited. Similar results are obtained with two other **amyloidogenic** substrates, mammalian **PrP** and .beta.-**amyloid** 1-42 peptide, but not with several control proteins. With a group of peptides that span the **PrP** protein sequence, those that produced the largest changes in CD spectra also caused the strongest inhibition of ATPase activity in Hsp104. Our observations suggest that (i) previously described genetic interactions between Hsp104 and [PSI+] are caused by

.alpha.2-macroglobulin (.alpha.2M), a proteinase inhibitor released in response to inflammatory stimuli, was identified as a strong and specific interactor of A.beta., utilizing this system. Direct evidence for this interaction was obtained by co-immunopptn. of .alpha.2M with A.beta. from the **yeast** cell, and by formation of SDS-resistant A.beta. complexes in polyacrylamide gels by using synthetic A.beta. and purified .alpha.2M. The assocn. of A.beta. with .alpha.2M and various purified **amyloid** binding proteins was assessed by employing a method measuring protein-protein interactions in liq. phase. The dissocn. const. by this technique for the .alpha.2M-A.beta. assocn. using labeled purified proteins was measured (Kd = 350 nM). Electron microscopy showed that a 1:8 ratio of .alpha.2M to A.beta. prevented fibril formation in soln.; the same ratio to A.beta. of another acute phase protein, .alpha.1-antichymotrypsin, was not active in preventing fibril formation in vitro. These results were corroborated by data obtained from an in vitro **aggregation** assay employing Thioflavine T. The interaction of .alpha.2M with A.beta. suggests new pathway(s) for the clearance of the sol. **amyloid** peptide.

L14 ANSWER 13 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:150568 CAPLUS
DN 128:281412
TI Twofold overexpression of human .beta.-**amyloid** precursor proteins in transgenic mice does not affect the neuromotor, cognitive, or neurodegenerative sequelae following experimental brain injury
AU Murai, Hisayuki; Pierce, Jean E. S.; Raghupathi, Ramesh; Smith, Douglas H.; Saatman, Kathryn E.; Trojanowski, John Q.; Lee, Virginia M.-Y.; Loring, Jeanne F.; Eckman, Chris; Younkin, Steven; McIntosh, Tracy K.
CS Department of Neurosurgery, University of Pennsylvania, Philadelphia, PA, 19104, USA
SO J. Comp. Neurol. (1998), 392(4), 428-438
CODEN: JCNEAM; ISSN: 0021-9967
PB Wiley-Liss, Inc.
DT Journal
LA English
AB By using transgenic mice that overexpress human P-**amyloid** precursor proteins (APPs) at levels twofold higher than endogenous APPs, following introduction of the human APP gene in a **yeast** artificial chromosome (YAC), we examd. the effects of controlled cortical impact (CCI) brain injury on neuromotor/cognitive dysfunction and the development of Alzheimer's disease (AD)-like neuropathol. Neuropathol. analyses included Nissl-staining and immunohistochem. to detect APPs, .beta.-**amyloid** (A.beta.), neurofilament proteins, and glial fibrillary acidic protein, whereas A.beta. levels were measured in brain homogenates from mice subjected to CCI and control mice by using a sensitive sandwich ELISA. Twenty APP-YAC transgenic mice and 17 wild type (WT) littermate controls were anesthetized and subjected to CCI (velocity, 5 m/s; deformation depth, 1 mm). Sham (anesthetized but uninjured) controls (n = 10 APP-YAC; n = 8 WT) also were studied. Motor function was evaluated by using rotarod, inclined-plane, and forelimb/hindlimb flexion tests. The Morris water maze was used to assess memory. Although CCI induced significant motor dysfunction and cognitive deficits, no differences were obsd. between brain-injured APP-YAC mice and WT mice at 24 h and 1 wk postinjury. By 1 wk postinjury, both cortical and hippocampal CA3 neuron loss as well as extensive astrogliosis were obsd. in all injured animals, suggesting that overexpression of human APPs exhibited no neuroprotective effects. Although AD-like pathol. (including **amyloid plaques**) was not obsd. in either sham or brain-injured animals, a significant decrease in brain concns. of only

AU Ohsawa, I.; Hirose, Y.; Ishiguro, M.; Imai, Y.; Ishiura, S.; Kohsaka,
CS National Institute of Neuroscience, Tokyo, Japan.
AV DNAL (442.8 B5236)
SO Biochemical and biophysical research communications, Aug 4, 1995. Vol. 213, No. 1. p. 52-58
Publisher: Orlando, Fla. : Academic Press.
CODEN: BBRCA9; ISSN: 0006-291X
NTE Includes references
CY Florida; United States
DT Article
FS U.S. Imprints not USDA, Experiment or Extension
LA English
AB The secreted form of amyloid precursor protein (APPs) including most of the extracellular domain of APP is released from the cell surface, suggesting physiological significance of Apps in vivo. We used the methylotrophic yeast Pichia pastoris as a host system for the production of recombinant Apps (rAPPs). Two rAPPss derived from isoforms of APP (APP695 and APP770) were secreted into the culture medium from the yeast, which carried cDNA encoding the N-terminal portion of APP under the control of a P. pastoris alcohol oxidase promoter. Like APPSs produced by the transfected COS-1 cells, the purified rAPPSs from yeast were shown to be biologically active in terms of neurite outgrowth of embryonic rat neocortical explants. These rAPPss could be valuable tools for
i

2 ANSWER 19 OF 23 BIOSIS COPYRIGHT 2000 BIOSIS DUPLICATE 8
AN 1995:41006 BIOSIS
DN PREV199598055306
TI Proteolytic processing and secretion of human beta-amyloid precursor protein in yeast: Evidence for a yeast secretase activity.
AU Zhang, Haiying; Komano, Hiroto; Fuller, Robert S.; Gandy, Samuel E.; Frail, Donald E. (1)
CS (1) Women's Health Res. Inst., Wyeth-Ayerst Res., 145 King of Prussia Rd., Radnor, PA 19807 USA
SO Journal of Biological Chemistry, (1994) Vol. 269, No. 45, pp. 27799-27802.
ISSN: 0021-9258.
DT Article
LA English
AB Human beta-**amyloid** precursor protein (APP), the transmembrane precursor of the Alzheimer's disease beta-**amyloid** peptide, was **expressed** in the **yeast** Saccharomyces cerevisiae by fusion to prepro-alpha-factor. From analysis of protease-deficient **yeast** strains, the fusion protein underwent partial processing by Kex2 protease to generate full-length APP and a fraction of the molecules were degraded in the vacuole. A soluble APP ectodomain fragment bearing lumenal but not cytosolic epitopes was released into the media, indicating cleavage by a "membrane protein-solubilizing proteinase" or "secretase" activity. Yeast cells contained a C-terminal APP fragment that co-migrated with authentic C-terminal fragment derived from alpha-secretase cleavage of full-length APP in human cells. The N-terminal sequence of immunoaffinity purified C-terminal APP fragment from yeast was identical to that reported in mammalian and insect cells. These results demonstrate the existence of a secretase activity in yeast. Furthermore, this yeast secretase activity may be related to an APP processing activity present in metazoan cells.

L2 ANSWER 9 OF 23 USPATFULL
AN 97:94081 USPATFULL
TI Human amyloid protein precursor homolog and kunitz-type inhibitor
IN Sprecher, Cindy A., Seattle, WA, United States
Foster, Donald C., Seattle, WA, United States
Norris, Kjeld E., Hellerup, Denmark
PA Zymogenetics, Inc., Seattle, WA, United States (U.S. corporation)
PI US 5677146 19971014
AI US 1995-424022 19950418 (8)
RLI Continuation of Ser. No. US 1993-155331, filed on 19 Nov 1993, now patented, Pat. No. US 5441931 And Ser. No. US 1992-985692, filed on 2 Dec 1992, now patented, Pat. No. US 5436153
DT Utility
EXNAM Primary Examiner: Furman, Keith C.
LREP Speckman, Ann W.; Sleath, Janet
CLMN Number of Claims: 6
ECL Exemplary Claim: 1,4
DRWN No Drawings
LN.CNT 1598
CAS INDEXING IS AVAILABLE FOR THIS PATENT.
AB The present invention provides isolated DNA molecules comprising a DNA segment encoding a novel human amyloid protein precursor homologue and novel Kunitz-type inhibitors. Also provided are DNA constructs comprising a first DNA segment encoding a novel human amyloid protein precursor homologue or a novel Kunitz-type inhibitor wherein said first DNA segment is operably linked to additional DNA segments required for the expression for the first DNA segment, as well as host cells containing such DNA constructs and methods for producing proteins from

L3 ANSWER 6 OF 8 CAPLUS COPYRIGHT 1999 ACS

AB . . . refs. Apolipoprotein (apo) E is assocd. with the two characteristic neuropathol. lesions of Alzheimer's disease--extracellular neuritic plaques representing deposits of **amyloid** beta (A.beta.) peptide and intracellular neurofibrillary tangles representing filaments of a microtubule-assocd. protein called tau. Incubation of the apoE4 isoform with the A.beta. peptide in **vitro** results in the formation of a dense, stable network of very long monofibrils, while incubation of apoE3 with the A.beta.. . . formed with the A.beta. peptide in the presence of apoE4 in vivo may impair the normal clearance process and enhance **plaque formation**. Alternatively or addnl., apoE may alter the cytoskeletal structure and function and, under certain conditions, may promote the formation of. . . authors studies have demonstrated that apoE3 and apoE4 exert differential effects on neuronal growth (i.e., neurite extension and branching) in **vitro**. When combined with a source of lipid, apoE3 stimulated neurite extension in peripheral nervous system neurons (dorsal root ganglia), whereas apoE4 **inhibited** it. Similar results were obtained with central nervous system neurons (murine neuroblastoma Neuro-2a cells). Addn. of free apoE3 or apoE4. . . within cell bodies and neurites to a greater extent than apoE4. Thus, apoE3 may facilitate cytoskeletal activity, whereas apoE4 may **inhibit** it, which would be detrimental during synaptic remodeling.

AN 1996:304677 CAPLUS

DN 125:6776

TI Apolipoprotein E: structure, function, and possible roles in Alzheimer's disease

AU Mahley, R.W.; Nathan, B.P.; Pitas, R.E.

CS Gladstone Institute of Cardiovascular Disease, University of California, San Francisco, CA, 94141-9100, USA

SO Ann. N. Y. Acad. Sci. (1996), 777(Neurobiology of Alzheimers Disease), 139-145
CODEN: ANYAA9; ISSN: 0077-8923

DT Journal; General Review

LA English

AB A review with 24 refs. Apolipoprotein (apo) E is assocd. with the two characteristic neuropathol. lesions of Alzheimer's disease--extracellular neuritic plaques representing deposits of **amyloid** beta (A.beta.) peptide and intracellular neurofibrillary tangles representing filaments of a microtubule-assocd. protein called tau. Incubation of the apoE4 isoform with the A.beta. peptide in **vitro** results in the formation of a dense, stable network of very long monofibrils, while incubation of apoE3 with the A.beta. peptide results in the formation of a less dense, less stable network. The more complex nature of the plaques formed with the A.beta. peptide in the presence of apoE4 in vivo may impair the normal clearance process and enhance **plaque formation**. Alternatively or addnl., apoE may alter the cytoskeletal structure and function and, under certain conditions, may promote the formation of the neurofibrillary tangles. The authors studies have demonstrated that apoE3 and apoE4 exert differential effects on neuronal growth (i.e., neurite extension and branching) in **vitro**. When combined with a source of lipid, apoE3 stimulated neurite extension in peripheral nervous system neurons (dorsal root ganglia), whereas apoE4 **inhibited** it. Similar results were obtained with central nervous system neurons (murine neuroblastoma Neuro-2a cells). Addn. of free apoE3 or apoE4 without .beta.-VLDL had no effect on neurite outgrowth. There was also differential accumulation of apoE3 and apoE4 by

the neuroblastoma cells: apoE3 accumulated within cell bodies and neurites to a greater extent than apoE4. Thus, apoE3 may facilitate cytoskeletal activity, whereas apoE4 may **inhibit** it, which would be detrimental during synaptic remodeling.

L3 ANSWER 7 OF 8 CAPLUS COPYRIGHT 1999 ACS
TI Selective **inhibition** of A.beta. fibril formation
AB The authors describe here an **inhibitor** of in **vitro** fibril formation, hexadecyl-N-methylpiperidinium (HMP) bromide, which is selective for the Alzheimer's disease peptide A.beta.. At 10 .mu.M, its IC50 for **inhibiting** A.beta. aggregation at pH 5.8, HMP bromide does not **inhibit** fibril formation by other amyloidogenic polypeptides nor does it affect the folding stability of the .beta.-sheet-rich Ig VL domain REI. In addn., small structural modifications of HMP bromide reduce or eliminate its ability to **inhibit** pH 5.8 aggregation of A.beta.. These indications of specificity, plus the ability of the mol. to **inhibit** A.beta. aggregation at concns. almost an order of magnitude below its crit. micelle concn., suggest a mechanism of **inhibition** other than micellar solubilization of A.beta.. HMP bromide is required in approx. a 1:1 stoichiometry for effective **inhibition** at pH 5.8. Although stoichiometric amts. of HMP bromide with respect to total A.beta. **inhibit** A.beta. fibril formation at pH 7.4, the mol. is incapable, at lower concns., of blocking the seeding of fibril formation. . . on A.beta. capable of binding amphipathic mols. such as HMP bromide and which, when occupied, precludes assembly of A.beta. into **amyloid** fibrils. Mols. that bind to this site with high specificity may prove to be useful therapeutic agents for preventing or retarding the cerebral **amyloid plaque formation** implicated in Alzheimer's disease pathol.
ST Abeta peptide fibril **inhibition** hexadecyl methylpiperidinium
IT Molecular structure-biological activity relationship
(fibril-**inhibiting**; selective **inhibition** of A.beta. fibril formation)
IT Proteins, specific or class
RL: BPR (Biological process); BIOL (Biological study); PROC (Process)
(**amyloid** A4, A.beta. peptide of, fibril formation-**inhibiting**; selective **inhibition** of A.beta. fibril formation)
IT Organelle
(fibril, selective **inhibition** of A.beta. fibril formation)
IT 1119-94-4, Dodecyltrimethylammonium bromide 1119-97-7, Myristyltrimethylammonium bromide 14933-09-6 41672-91-7
RL: BAC (Biological activity or effector, except adverse); BIOL (Biological study)
(selective **inhibition** of A.beta. fibril formation)
AN 1996:130523 CAPLUS
DN 124:223914
TI Selective **inhibition** of A.beta. fibril formation
AU Wood, Stephen J.; MacKenzie, Laurie; Maleeff, Beverly; Hurle, Mark R.; Wetzel, Ronald
CS Dep. Macromol. Sci., SmithKline Beecham Pharm., King of Prussia, PA, 19406, USA
SO J. Biol. Chem. (1996), 271(8), 4086-92
CODEN: JBCHA3; ISSN: 0021-9258
DT Journal
LA English
AB The authors describe here an **inhibitor** of in **vitro** fibril formation, hexadecyl-N-methylpiperidinium (HMP) bromide, which is selective for the Alzheimer's disease peptide A.beta.. At 10 .mu.M, its IC50 for **inhibiting** A.beta. aggregation at pH 5.8, HMP bromide does not **inhibit** fibril formation by other amyloidogenic polypeptides nor does it affect the folding stability of the .beta.-sheet-rich Ig VL domain REI. In addn., small structural

modifications of HMP bromide reduce or eliminate its ability to **inhibit** pH 5.8 aggregation of A.beta.. These indications of specificity, plus the ability of the mol. to **inhibit** A.beta. aggregation at concns. almost an order of magnitude below its crit. micelle concn., suggest a mechanism of **inhibition** other than micellar solubilization of A.beta.. HMP bromide is required in approx. a 1:1 stoichiometry for effective **inhibition** at pH 5.8. Although stoichiometric amts. of HMP bromide with respect to total A.beta. **inhibit** A.beta. fibril formation at pH 7.4, the mol. is incapable, at lower concns., of blocking the seeding of fibril formation by small amts. of added A.beta. fibrils. The results suggest the existence of a binding surface on A.beta. capable of binding amphipathic mols. such as HMP bromide and which, when occupied, precludes assembly of A.beta. into **amyloid** fibrils. Mols. that bind to this site with high specificity may prove to be useful therapeutic agents for preventing or retarding the cerebral **amyloid plaque formation** implicated in Alzheimer's disease pathol.

L13 ANSWER 4 OF 14 CAPLUS COPYRIGHT 1999 ACS
AN 1998:31443 CAPLUS
DN 128:84380
TI Screening compounds for the ability to alter the **production** of amyloid-.beta. peptide (x .gtoreq. 41)
IN Citron, Martin; Selkoe, Dennis J.; Seubert, Peter A.; Schenk, Dale
PA Athena Neurosciences, Inc., USA; Brigham and Women's Hospital; Citron, Martin; Selkoe, Dennis J.; Seubert, Peter A.; Schenk, Dale
SO PCT Int. Appl., 86 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9748983	A1	19971224	WO 97-US10601	19970618
	W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, HU, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM				
	RW: GH, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG				
	CA 2258348	AA	19971224	CA 97-2258348	19970618
	AU 9735727	A1	19980107	AU 97-35727	19970618
	EP 906577	A1	19990407	EP 97-932208	19970618
	R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI US 96-665649 19960618
WO 97-US10601 19970618
AB This invention provides methods of screening compds. for their ability to alter the prodn. of A.beta. (x .gtoreq. 41) alone or in combination with A.beta. (x .ltoreq. 40). The methods involve administering compds. to **cells**, specifically measuring the amts. of A.beta. (x .ltoreq. 40) and A.beta. (x .gtoreq. 41) produced by the **cells**, and comparing these amts. to that produced by the **cells** without administration of the compds.

L13 ANSWER 8 OF 14 CAPLUS COPYRIGHT 1999 ACS
AN 1995:328464 CAPLUS
DN 122:98802
TI The introduction and expression of large genomic sequences into animal **cells** using yeast artificial chromosomes and the development of transgenic animals
IN Gearhart, John D.; Lamb, Bruce T.
PA Johns Hopkins University, USA
SO PCT Int. Appl., 61 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI	WO 9423049	A2	19941013	WO 94-US3619	19940401
	WO 9423049	A3	19950105		
	W: CA, JP				
	RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE				

PRAI US 93-42390 19930402
AB This invention provides a method for the efficient introduction of cloned,

very high mol. wt. **DNA** into the germline of mice, whereby large genes can be expressed appropriately in transgenic mice. The . **beta.-amyloid** precursor protein (APP) is known to be a complex gene consisting of 18 exons with total size ests. greater than 170 kb encoding three major RNA splicing forms. A neomycin resistance cassette is introduced into one of the arms of a 650 kb yeast artificial chromosome (YAC) which contains the entire rearranged APP gene within 400 kb. Following gel purifn., the YAC is introduced into embryonic stem (ES) **cells** by lipid-mediated transfection using Lipofectin.RTM.. Neomycin resistant ES lines are isolated with the human APP gene stably integrated in an unrearranged state and expressing properly initiated and spliced full length human APP mRNA and APP human protein. Mouse chimeras generated from these ES lines transmit the YAC to their offspring, generating novel APP YAC transgenic mice. These transgenic mice express human APP gene **products** at significant levels in brain and peripheral tissues that mirror the expression of endogenous mouse APP gene **products**. This procedure will have great utility for transgenic studies of gene expression involving large genes and gene complexes.

L13 ANSWER 10 OF 14 CAPLUS COPYRIGHT 1999 ACS
AN 1994:214527 CAPLUS
DN 120:214527
TI Screen for Alzheimer's disease therapeutics based on **.beta.-amyloid production**
IN Yankner, Bruce A.
PA Children's Medical Center Corp., USA
SO PCT Int. Appl., 18 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9401772	A1	19940120	WO 93-US6589	19930713

W: JP
RW: AT, BE, CH, DE, DK, ES, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE
PRAI US 92-912531 19920713
AB Normal mammalian **cells** [which generally express amyloid precursor protein (APP)-encoding **DNA**] process the APP **product** so as to provide detectable extracellular levels of . **beta.-amyloid** peptide. This finding enables an important and long sought screening for compds. that may be suitable therapeutics to treat, prevent, control, or lessen the severity of Alzheimer's disease, as a result of their ability to influence the prodn. of extracellular **.beta.-amyloid** peptide. The therapeutic capacity of a candidate compd. for treating Alzheimer's disease is assessed by: (a) providing a population of mammalian **cells** which expresses APP, and which produces extracellular . **beta.-amyloid** peptide; (b) culturing that population in a culture medium comprising the candidate compd.; and (c) measuring the extracellular amt. of **.beta.-amyloid** peptide so as to det. the effect (if any) of the candidate compd. on the extracellular amt. of **.beta.-amyloid** peptide. Detection of **.beta .-amyloid** peptide secreted by COS-1 **cells** transfected with the APP expression plasmid was demonstrated.

L13 ANSWER 13 OF 14 CAPLUS COPYRIGHT 1999 ACS
AN 1991:158091 CAPLUS
DN 114:158091
TI The Drosophila transcript encoded by the **.beta.-amyloid** protein precursor-like gene is restricted to the nervous system
AU Martin-Morris, Linda E.; White, Kalpana

CS Dep. Biol., Brandeis Univ., Waltham, MA, 02254, USA
SO Development (Cambridge, UK) (1990), 110(1), 185-95
CODEN: DEVPED; ISSN: 0950-1991
DT Journal
LA English
AB A Drosophila .**beta**.-**amyloid** protein precursor-like (Appl) gene was delineated and its pattern of expression was analyzed. Appl defines a new locus within the 1B division of the X-chromosome, a region previously shown to be important for neural development. The genomic limits of the Appl gene were defined by mapping of the Appl cDNAs.
The Appl transcript spans .apprx.38 kb of genomic **DNA**. Genomic regions surrounding the first 2 exons were sequenced. The first exon contains 78 nucleotides of the coding sequence and is sepd. from the second exon by a .apprx.21-kb intron. The second exon is 171 nucleotides long and is sepd. from the third exon by a .apprx.7-kb intron. In situ RNA localization data is presented that demonstrate that the Appl transcript is found in post-mitotic neurons in all developmental stages, in the central and peripheral nervous systems. Within the nervous system,
transcripts are not obsd. in neuroblasts, newly generated neurons, and .gtoreq.1 class of presumed glial **cells**. The temporal and spatial specificity of Appl expression suggests that the gene **product** has a function that is common to most neurons. Appl cDNA predicts an 886-amino acid polypeptide that exhibits strong sequence similarity to the human .**beta**.-**amyloid** protein precursor (APP) (Rosen et al. 1989). In this paper, Appl gene expression is compared with the pattern of expression of the .**beta**.-**amyloid** protein precursor (APP) gene in mammals. Furthermore, it is suggested that during evolution, a neural-specific function encoded by the APP gene has been selectively maintained.

=> d bib ab 12

L13 ANSWER 12 OF 14 CAPLUS COPYRIGHT 1999 ACS
AN 1992:402142 CAPLUS
DN 117:2142
TI Repression of the .**beta**.-**amyloid** gene in a Hox-3.1-producing cell line
AU Violette, Sheila M.; Shashikant, Cooduvalli S.; Salbaum, J. Michael; Belting, Heinz Georg; Wang, Jean C. H.; Ruddle, Frank H.
CS Dep. Biol., Yale Univ., New Haven, CT, 06511, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1992), 89(9), 3805-9
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB Mammalian homeobox genes are widely expressed in the developing central nervous system and are postulated to control developmental processes by regulating gene expression at the transcriptional level. In vitro studies
have identified consensus **DNA** sequences that contain an ATTA core as sites for interaction with homeodomain proteins. Such elements have been found in the upstream regulatory region of the gene encoding .**beta**.-**amyloid** precursor protein, which is assocd. with the neurol. disorder Alzheimer disease. As the .**beta**.-**amyloid** precursor protein gene is also expressed in the developing central nervous system and appears to play a role in cellular regulatory processes, the authors have examd. the possibility that a homeobox gene **product** can regulate its transcription. The authors demonstrate by Northern blot analyses and transfection expts. that the expression of the .**beta**.-**amyloid** precursor protein gene is decreased in cultured **cells** expressing the mouse homeobox gene Hox-3.1.

L3 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1999:388321 CAPLUS
DN 131:13980
TI Methods using a **yeast** cell system for identifying factors controlling **amyloid** protein aggregation
IN Lindquist, Susan
PA Arch Development Corporation, USA
SO PCT Int. Appl., 64 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9929891	A1	19990617	WO 1998-US26113	19981209

W: AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

PRAI US 1997-69168 19971209
US 1998-84824 19980508
AB The present invention provides a **yeast** cell-based system for detg. factors that control the folding of **amyloid** proteins of diverse origins. Further, the present invention provides methods of using such a system to screen for reagents that effect **amyloid** formation, a process that is integral to several devastating human diseases including Creutzfeld-Jacob disease (CJD), fatal familial insomnia (FFI), Gertsmann-Straussler-Scheinker (GSS) syndrome, and kuru. The system of the present invention provides a rapid screening system to quickly and cheaply identify reagents that effect the folding and aggregation properties of the target protein. CD studies provided evidence for the direct interaction of Hsp104 with Sup35 of **yeast** and with PrP. Both Sup35 and PrP inhibit the ATPase activity of Hsp104.

L3 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1999:113882 CAPLUS
DN 130:193967
TI Novel method of detecting **amyloid**-like fibrils or protein aggregates using filters for disease diagnosis and inhibitor identification
IN Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian
PA Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany
SO PCT Int. Appl., 56 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9906838	A2	19990211	WO 1998-EP4810	19980731

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL,

PT, SE

AB The present invention relates to methods of detecting the presence of detergent- or urea-insol. **amyloid**-like fibrils or protein aggregates on filters. Preferably, the fibrils or aggregates are indicative of a disease, preferably of a neurodegenerative disease such as Alzheimer's disease or Huntington's disease. In addn., the present invention relates to inhibitors identified by the method of the invention, to pharmaceutical compns. comprising the inhibitors and to diagnostic compns. useful for the investigation of the **amyloid**-like fibrils or aggregates. Protein samples were treated with SDS and filtered through cellulose acetate membranes in a BRL dot blot filtration unit. The filters were washed with SDS soln., blocked, treated with antibody, labeled with secondary antibody-peroxidase conjugate or other detection system, and quantified.

L3 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1999:113797 CAPLUS
DN 130:166800
TI Soluble fusion proteins of aggregate-forming proteins and the study of diseases associated with protein aggregate formation
IN Wanker, Erich; Lehrach, Hans; Scherzinger, Eberhard; Bates, Gillian
PA Max-Planck-Gesellschaft zur Forderung der Wissenschaften e.V., Germany
SO PCT Int. Appl., 62 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9906545	A2	19990211	WO 1998-EP4811	19980731

W: CA, JP, US
RW: AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE

N/A

AB Fusion proteins of aggregate-forming proteins and solubilizing peptides are described for use in elucidating the mechanism, onset or progress of diseases assocd. with the formation of **amyloid**-like fibrils or protein aggregates. The method is for use in the study of neurol. diseases such as Huntington's and Alzheimer's. The fusion proteins can also be used to screen for **inhibitors** of **aggregation** that may be of therapeutic use. Genes for a series of fusion proteins polyglutamine repeat expansion variants (20, 30, or 51 glutamine repeats) of huntingtin and glutathione-S-transferase were constructed by std. methods and manufd. in Escherichia coli using a hexahistidine for affinity purifn. The fusion proteins were sol. but cleavage of the 51 glutamine repeat variant (HD51) with trypsin led to the formation of insol. aggregates of the huntingtin. HD51 aggregated in vitro to form **amyloid**-like birefringent fibrils after liberation by trypsin cleavage, but the shorter repeat variants HD20 and HD30 did not do so. Similar effects were seen in vivo in COS-1 cells.

L9 ANSWER 1 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1998:688033 CAPLUS
DN 130:35563
TI Mechanism of inhibition of .PSI.+ **prion** determinant propagation by a mutation of the N-terminus of the **yeast Sup35** protein
AU Kochneva-Pervukhova, Natalia V.; Paushkin, Sergey V.; Kushnirov, Vitaly V.; Cox, Brian S.; Tuite, Mick F.; Ter-Avanesyan, Michael D.
CS Cardiology Research Center, Institute of Experimental Cardiology, Moscow, 121552, Russia
SO EMBO J. (1998), 17(19), 5805-5810
CODEN: EMJODG; ISSN: 0261-4189
PB Oxford University Press
DT Journal
LA English
AB The **SUP35** gene of Saccharomyces cerevisiae encodes the polypeptide chain release factor eRF3. This protein (also called Sup35p) is thought to be able to undergo a heritable conformational switch, similarly to mammalian **prions**, giving rise to the cytoplasmically inherited .PSI.+ determinant. A dominant mutation (PNM2 allele) in the **SUP35** gene causing a Gly58.fwdarw.Asp change in the Sup35p N-terminal domain eliminates .PSI.+. Here we obsd. that the mutant Sup35p can be converted to the **prion**-like form in vitro, but such conversion proceeds slower than that of wild-type Sup35p. The overexpression of mutant Sup35p induced the de novo appearance of .PSI.+ cells contg. the **prion**-like form of mutant Sup35p, which was able to transmit its properties to wild-type Sup35p both in vitro and in vivo. Our data indicate that this .PSI.+-eliminating mutation does not alter the initial binding of Sup35p mols. to the Sup35p .PSI.+-specific **aggregates**, but rather **inhibits** its subsequent **prion**-like rearrangement and/or binding of the next Sup35p mol. to the growing **prion**-like Sup35p aggregate.

L9 ANSWER 2 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1997:275947 CAPLUS
DN 126:327804
TI Interaction between **yeast** Sup45p (eRF1) and Sup35p (eRF3) polypeptide chain release factors: implications for **prion** -dependent regulation
AU Paushkin, Sergey V.; Kushnirov, Vitaly V.; Smirnov, Vladimir N.; Ter-Avanesyan, Michael D.
CS Inst. Exp. Cardiol., Cardiol. Res. Cent., Moscow, 121552, Russia
SO Mol. Cell. Biol. (1997), 17(5), 2798-2805
CODEN: MCEBD4; ISSN: 0270-7306
PB American Society for Microbiology
DT Journal
LA English
AB The SUP45 and **SUP35** genes of Saccharomyces cerevisiae encode polypeptide chain release factors eRF1 and eRF3, resp. It has been suggested that the **Sup35** protein (Sup35p) is subject to a heritable conformational switch, similar to mammalian **prions**, thus giving rise to the non-Mendelian [PSI+] nonsense suppressor determinant. In a [PSI+] state, Sup35p forms high-mol.-wt. **aggregates** which may **inhibit** Sup35p activity, leading to the [PSI+] phenotype. Sup35p is composed of the N-terminal domain (N) required for [PSI+] maintenance, the presumably nonfunctional middle region (M), and the C-terminal domain (C) essential for translation termination. In this study, we obsd. that the N domain, alone or as a part of larger fragments, can form aggregates in [PSI+] cells. Two sites for Sup45p binding were found within Sup35p: one is formed by the N and M

domains, and the other is located within the C domain. Similarly to Sup35p, in [PSI+] cells Sup45p was found in aggregates. The aggregation of Sup45p is caused by its binding to Sup35p and was not obsd. when the aggregated Sup35p fragments did not contain sites for Sup45p binding. The incorporation of Sup45p into the **aggregates** should **inhibit** its activity. The N domain of Sup35p, responsible for its aggregation in [PSI+] cells, may thus act as a repressor of another polypeptide chain release factor, Sup45p. This phenomenon represents a novel mechanism of regulation of gene expression at the posttranslational level.

L9 ANSWER 3 OF 3 CAPLUS COPYRIGHT 1999 ACS
AN 1996:394350 CAPLUS
DN 125:53283
TI Propagation of the **yeast prion**-like [psi+] determinant is mediated by oligomerization of the **SUP35**-encoded polypeptide chain release factor
AU Kushniin, Sergey V.; Kushnirov, Vitaly V.; Smirnov, Vladimir N.; Ter-Avanesyan, Michael D.
CS Cardiology Res. Center, Inst. Experimental Cardiology, Moscow, 121552, Russia
SO EMBO J. (1996), 15(12), 3127-3134
CODEN: EMJODG; ISSN: 0261-4189
DT Journal
LA English
AB The Sup35p protein of **yeast** Saccharomyces cerevisiae is a homolog of the polypeptide chain release factor 3 (eRF3) of higher eukaryotes. It has been suggested that this protein may adopt a specific self-propagating conformation, similar to mammalian **prions**, giving rise to the [psi+] nonsense suppressor determinant, inherited in a non-Mendelian fashion. Here, the authors present data confirming the **prion**-like nature of [psi+]. They show that Sup35p mols. interact with each other through their N-terminal domains in [psi+], but not [psi-], cells. This interaction is crit. for [psi+] propagation, since its disruption leads to a loss of [psi+]. Similarly to mammalian **prions**, in [psi+] cells Sup35p forms high mol. wt. aggregates, accumulating most of this protein. The **aggregation** **inhibits** Sup35p activity, leading to a [psi+] nonsense-suppressor phenotype. N-terminally altered Sup35p mols. are unable to interact with the [psi+] Sup35p isoform, remain sol. and improve the translation termination in [psi+] strains, thus causing an antisuppressor phenotype. The overexpression of Hsp104p chaperone protein partially solubilizes Sup35p aggregates in the [psi+] strain, also causing an antisuppressor phenotype. The authors propose that Hsp104p plays a role in establishing stable [psi+] inheritance by splitting up Sup35p aggregates and thus ensuring equidistribution of the **prion**-like Sup35p isoform to daughter cells at cell divisions.

L14 ANSWER 1 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1999:409775 CAPLUS
TI Equilibrium Folding Properties of the **Yeast Prion** Protein Determinant Ure2
AU Perrett, Sarah; Freeman, Samantha J.; Butler, P. Jonathan G.; Fersht, Alan R.
CS Centre for Protein Engineering, Department of Chemistry, University of Cambridge, Cambridge, CB2 1EW, UK
SO J. Mol. Biol. (1999), 290(1), 331-345
CODEN: JMOBAK; ISSN: 0022-2836
PB Academic Press
DT Journal
LA English
AB The **yeast** non-Mendelian factor [**URE3**] propagates by a **prion**-like mechanism, involving **aggregation** of the chromosomally encoded protein Ure2. The [**URE3**] phenotype is equiv. to loss of function of Ure2, a protein involved in regulation of nitrogen metab. The **prion**-like behavior of Ure2 in vivo is dependent on the first 65 amino acid residues of its N-terminal region which contains a highly repetitive sequence rich in asparagine. This region has been termed the **prion**-detg. domain (PrD). Removal of as little as residues 2-20 of the protein is sufficient to prevent occurrence of the [**URE3**] phenotype. Removal of the PrD does not affect the regulatory activity of Ure2. The C-terminal portion of the protein has homol. to glutathione S -transferases, which are dimeric proteins. We have produced the Ure2 protein to high yield in Escherichia coli from a synthetic gene. The recombinant purified protein is shown to be a dimer. The stability, folding and oligomeric state of Ure2 and a series of N-terminally truncated or deleted variants were studied and compared. The stability of Ure2, .DELTA.GD-N, H2O, detd. by chem. denaturation and monitored by fluorescence, is 12.1(.+-.0.4) kcal mol-1at 25 .degree.C and pH 8.4. A range of structural probes show a single, coincident unfolding transition, which is invariant over a 550-fold change in protein concn. The stability is the same within error for Ure2 variants lacking all or part of the **prion**-detg. domain. The data indicate that in the folded protein the PrD is in an unstructured conformation and does not form specific intra- or intermol. interactions at micromolar protein concns. This suggests that the C-terminal domain may stabilize the PrD against **prion** formation by steric means, and implies that the PrD does not induce **prion** formation by altering the thermodn. stability of the folded protein. (c) 1999 Academic Press.

L14 ANSWER 2 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1999:205819 CAPLUS
DN 131:15387
TI Characterization of the interaction domains of Ure2p, a **prion** -like protein of **yeast**
AU Fernandez-Bellot, Eric; Guillemet, Elisabeth; Baudin-Baillieu, Agnbs; Gaumer, Sebastien; Komar, Anton A.; Cullin, Christophe
CS Centre de Genetique Moleculaire du C.N.R.S., Laboratoire Propre Associ, Universite Pierre-et-Marie-Curie, Gif-sur-Yvette, 91190, Fr.
SO Biochem. J. (1999), 338(2), 403-407
CODEN: BIJOAK; ISSN: 0264-6021
PB Portland Press Ltd.
DT Journal

LA English
AB In the **yeast** Saccharomyces cerevisiae, the non-Mendelian inherited genetic element [**URE3**] behaves as a **prion**. A hypothesis has been put forward which states that [**URE3**] arises spontaneously from its cellular isoform Ure2p (the product of the URE2 gene), and propagates through interactions of the N-terminal domain of the protein, thus leading to its **aggregation** and loss of function. In the present study, various N- and C-terminal deletion mutants of Ure2p were constructed and their cross-interactions were tested in vitro and in vivo using affinity binding and a two-hybrid anal. We show that the self-interaction of the protein is mediated by at least two domains, corresponding to the first third of the protein (the so-called **prion**-forming domain) and the C-terminal catalytic domain.

L14 ANSWER 3 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1999:197688 CAPLUS
TI The **yeast** non-Mendelian factor [ETA+] is a variant of [PSI+], a **prion**-like form of release factor eRF3
AU Zhou, Ping; Derkatch, Irina L.; Uptain, Susan M.; Patino, Maria M.; Lindquist, Susan; Liebman, Susan W.
CS Laboratory for Molecular Biology, Department of Biological Sciences, University of Illinois at Chicago, Chicago, IL, 60607, USA
SO EMBO J. (1999), 18(5), 1182-1191
CODEN: EMJODG; ISSN: 0261-4189
PB Oxford University Press
DT Journal
LA English
AB The **yeast** non-Mendelian factor [ETA+] is lethal in the presence of certain mutations in the **SUP35** and SUP45 genes, which code for the translational release factors eRF3 and eRF1, resp. One such mutation, **sup35**-2, is now shown to contain a UAG stop codon prior to the essential region of the gene. The non-Mendelian inheritance of [ETA+] is reminiscent of the **yeast** [PSI+] element, which is due to a self-propagating conformation of Sup35p. Here we show that [ETA+] and [PSI+] share many characteristics. Indeed, like [PSI+], the maintenance of [ETA+] requires the N-terminal region of Sup35p and depends on an appropriate level of the chaperone protein Hsp104. Moreover, [ETA+] can be induced de novo by excess Sup35p, and [ETA+] cells have a weak nonsense suppressor phenotype characteristic of weak [PSI+]. We conclude that [ETA+] is actually a weak, unstable variant of [PSI+]. We find that although some Sup35p **aggregates** in [ETA+] cells, more Sup35p remains sol. in [ETA+] cells than in isogenic strong [PSI+] cells. Our data suggest that the amt. of sol. Sup35p dets. the strength of translational nonsense suppression assocd. with different [PSI+] variants.

L14 ANSWER 4 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1999:152160 CAPLUS
DN 130:277604
TI The PNM2 mutation in the **prion** protein domain of **SUP35** has distinct effects on different variants of the [PSI+] **prion** in **yeast**
AU Derkatch, Irina L.; Bradley, Michael E.; Zhou, Ping; Liebman, S. W.
CS Department of Biological Sciences, Laboratory for Molecular Biology, University of Illinois at Chicago, 900 S. Ashland Avenue, Chicago, IL, 60304, USA
SO Curr. Genet. (1999), 35(2), 59-67
CODEN: CUGED5; ISSN: 0172-8083
PB Springer-Verlag
DT Journal
LA English
AB We have previously described different variants of the **yeast** **prion** [PSI+] that can be obtained and maintained in the same

genetic background. These [PSI+] variants, which differ in the efficiency of nonsense suppression, mitotic stability and the efficiency of curing by guanidine hydrochloride, may correspond to different [PSI+] **prion** conformations of Sup35p or to different types of **prion** **aggregates**. Here we investigate the effects of overexpressing a mutant allele of **SUP35** and find different effects on weak and strong [PSI+] variants: the suppressor phenotype of weak [PSI+] factors is increased, whereas the suppressor effect of strong [PSI+] factors is reduced. The **SUP35** mutation used was originally described as a "Psi no more" mutation (PNM2) because it caused loss of [PSI+]. However, none of the [PSI+] variants in the strains used in our study were cured by PNM2. Indeed, when overexpressed, PNM2 induced the de novo appearance of both weak and strong [PSI+] variants with approx. the same efficiency as the overexpressed wild-type **SUP35** allele. Our data suggest that the change in the region of oligopeptide repeats in the Sup35p N-terminus due to the PNM2 mutation modifies, but does not impair, the function of the **prion** domain of Sup35p.

L14 ANSWER 5 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1999:150897 CAPLUS
DN 130:334295
TI The [**URE3**] **prion** is an **aggregated** form of Ure2p that can be cured by overexpression of Ure2p fragments
AU Edskes, Herman K.; Gray, Vaughn T.; Wickner, Reed B.
CS Laboratory of Biochemistry and Genetics, National Institute of Diabetes and Digestive and Kidney Diseases, National Institutes of Health, Bethesda, MD, 20892-0830, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1999), 96(4), 1498-1503
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB The [**URE3**] nonchromosomal genetic element is a **prion** of Ure2p, a regulator of nitrogen catabolism in Saccharomyces cerevisiae. Ure2p1-65 is the **prion** domain of Ure2p, sufficient to propagate [**URE3**] in vivo. The authors show that full length Ure2p-green fluorescent protein (GFP) or a Ure2p1-65-GFP fusion protein is **aggregated** in cells carrying [**URE3**] but is evenly distributed in cells lacking the [**URE3**] **prion**. This indicates that [**URE3**] involves a self-propagating **aggregation** of Ure2p. Overexpression of Ure2p1-65 induces the de novo appearance of [**URE3**] by 1,000-fold in a strain initially [ure-o], but cures [**URE3**] from a strain initially carrying the [**URE3**] **prion**. Overexpression of several other fragments of Ure2p or Ure2-GFP fusion proteins also efficiently cures the **prion**. The authorssuggest that incorporation of fragments or fusion proteins into a putative [**URE3**] "crystal" of Ure2p poisons its propagation.

L14 ANSWER 6 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:805473 CAPLUS
DN 130:193994
TI [**URE3**] and [PSI] are **prions** of **yeast** and evidence for new fungal **prions**
AU Masison, Daniel C.; Edskes, Herman K.; Maddelein, Marie-Lise; Taylor, Kimberly L.; Wickner, Reed B.
CS National Institutes of Health, Bethesda, MD, 20892-0830, USA
SO Prions (1999), 193-212. Editor(s): Harris, David A. Publisher: Horizon Scientific Press, Norfolk, UK.
CODEN: 67CGAH
DT Conference; General Review
LA English

AB A review and discussion with 75 refs. [**URE3**] and [PSI] are two non-Mendelian genetic elements discovered over 25 yr ago and never assigned to a nucleic acid replicon. Their genetic properties suggested that they are **prions**, altered self-propagating forms of Ure2p and Sup35p, resp., that cannot properly carry out the normal functions of these proteins. Ure2p is partially protease-resistant in [**URE3**] strains, and Sup35p is **aggregated** specifically in [PSI] strains supporting this idea. Overexpression of Hsp 104 cures [PSI], as does the absence of this protein, suggesting that the **prion** change of Sup35p in [PSI] strains is **aggregation**. Strains of [PSI], analogous to those described for scrapie, have now been described as well as an in vitro system for [PSI] propagation. Recently, two new potential **prions** have been described, one in **yeast** and the other in the filamentous fungus, Podospora.

L14 ANSWER 7 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:624763 CAPLUS
DN 129:328186
TI Processing of the Alzheimer's disease **amyloid** precursor protein in Pichia pastoris: Immunodetection of .alpha.-, .beta.-, and .gamma.-secretase products
AU Le Brocque, Darren; Henry, Anna; Cappai, Roberto; Li, Qiao-Xin; Tanner, Jane E.; Galatis, Denise; Gray, Carol; Holmes, Steven; Underwood, John R.;
Beyreuther, Konrad; Masters, Colin L.; Evin, Genevieve
CS Department of Pathology and The Mental Health Research Institute, The University of Melbourne, Parkville, 3052, Australia
SO Biochemistry (1998), 37(42), 14958-14965
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB .beta.A4 (A.beta.) **amyloid** peptide, a major component of Alzheimer's disease (AD) **plaques**, is a proteolytic product of the **amyloid** precursor protein (APP). Endoproteases, termed .beta.- and .gamma.-secretase, release resp. the N- and C-termini of the peptide. APP default secretion involves cleavage within the .beta.A4 domain by .alpha.-secretase. To study the conservation of APP processing in lower eukaryotes, the **yeast** Pichia pastoris was transfected with human APP695 cDNA. In addn. to the full-length integral transmembrane protein found in the cell lysate, sol./secreted APP (sAPP) was detected in the culture medium. Most sAPP comprised the N-terminal moiety of .beta.A4 and corresponds to sAPP.alpha., the product of .alpha.-secretase. The culture medium also contained minor secreted forms
detected by a monoclonal antibody specific for sAPP.beta. (the ectodomain released by .beta.-secretase cleavage). Anal. of the cell lysates with specific antibodies also detected membrane-assocd. C-terminal fragments corresponding to the products of .alpha. and .beta. cleavages. Moreover, immunopptn. of the culture medium with three antibodies directed at distinct epitopes of the .beta.A4 domain yielded a 4 kDa product with the same electrophoretic mobility as .beta.A4 synthetic peptide. These results suggest that the .alpha.-, .beta.-, and .gamma.-secretase cleavages are conserved in **yeast** and that P. pastoris may offer an alternative to mammalian cells to identify the proteases involved in the generation of AD .beta.A4 **amyloid**.

L14 ANSWER 8 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:588463 CAPLUS
DN 129:287704
TI **Amyloid** fibers of **Sup35** support a **prion**-like mechanism of inheritance in **yeast**
AU Lindquist, S.; DebBurman, S. K.; Glover, J. R.; Kowal, A. S.; Liu, J.-J.; Schirmer, E. C.; Serio, T. R.
CS Howard Hughes Medical Institute, The Department of Molecular Genetics and Cell Biology, The University of Chicago, Chicago, IL, 60637, USA

SO Biochem. Soc. Trans. (1998), 26(3), 486-490
CODEN: BCSTB5; ISSN: 0300-5127
PB Portland Press Ltd.
DT Journal
LA English
AB Demonstration that the in vitro **aggregation** of the **amyloidogenic** protein **Sup35** proceeds by a highly ordered, self-seeded mechanism provides strong support for the expansion of the **prion** hypothesis to include the cytosolic transmission of a phenotypic trait in **yeast**.

L14 ANSWER 9 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:549707 CAPLUS
DN 129:340428
TI C-terminal truncation of the **Sup35** protein increases the frequency of de novo generation of a **prion**-based [PSI+] determinant in Saccharomyces cerevisiae
AU Kochneva-Pervukhova, N. V.; Poznyakovski, A. I.; Smirnov, V. N.; Ter-Avanesyan, M. D.
CS Cardiology Research Center, Institute of Experimental Cardiology, Moscow, 121552, Russia
SO Curr. Genet. (1998), 34(2), 146-151
CODEN: CUGED5; ISSN: 0172-8083
PB Springer-Verlag
DT Journal
LA English
AB The **yeast** non-Mendelian [PSI+] determinant is presumed to be the manifestation of the **aggregated prion**-like state of the **Sup35** protein. Plasmid-mediated amplification of the **SUP35** gene greatly increases the frequency of Sup35p transition to this **prion**-like state. Here we show that the 3'-deletions of plasmid **SUP35**, leading to the C-terminal truncation of Sup35p, further increase the frequency of [PSI+] induction despite a marked decrease in Sup35p expression levels. The data suggest that the presence of Sup35p N-terminal proteolytic fragments can cause [PSI+] appearance in wild-type **yeast** cells.

L14 ANSWER 10 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:483415 CAPLUS
DN 129:258877
TI Subcellular localization of the Alzheimer's disease **amyloid** precursor protein and derived polypeptides expressed in a recombinant **yeast** system
AU Culvenor, Janetta G.; Henry, Anna; Hartmann, Tobias; Evin, Genevieve; Galatis, Denise; Friedhuber, Anna; Jayasena, U. L. H. Rajiv; Underwood, John R.; Beyreuther, Konrad; Masters, Colin L.; Cappai, Roberto
CS Department of Pathology, The University of Melbourne, Parkville, 3052, Australia
SO Amyloid (1998), 5(2), 79-89
CODEN: AIJIET; ISSN: 1350-6129
PB Parthenon Publishing Group
DT Journal
LA English
AB Different isoforms and derived polypeptides of the Alzheimer's disease **amyloid** protein precursor (A.beta.PP) have been expressed in the **yeast** Pichia pastoris. The expression characteristics of the different A.beta.PP polypeptides were studied by post-embedding immunogold electron microscopy with various A.beta.PP antibodies. The site of intracellular expression could be readily identified with specific antibodies. Full length A.beta.PP was expressed in assocn. with the nuclear membrane and the endoplasmic reticulum. Secretory derivs. of A.beta.PP were localized in membrane-bound secretory vesicles. A construct encoding two copies of .beta.A4[1-42] linked head-to-tail (.beta.A4duplex) accumulated as irregular dense cytoplasmic and intranuclear inclusions which reacted with all .beta.A4 antibodies tested.

direct interaction between Hsp104 and **Sup35**; (ii) **Sup35** and **PrP**, the determinants of the **yeast** and mammalian **prions**, resp., share structural features that lead to a specific interaction with Hsp104; and (iii) these interactions couple a change in structure to the ATPase activity of Hsp104.

L14 ANSWER 18 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1997:483625 CAPLUS
DN 127:187161
TI In vitro propagation of the **prion**-like state of **yeast** **Sup35** protein
AU Paushkin, Sergey V.; Kushnirov, Vitaly V.; Smirnov, Vladimir N.; Ter-Avanesyan, Michael D.
CS Inst. Experimental Cardiology, Cardiology Res. Cent., Moscow, 121552, Russia
SO Science (Washington, D. C.) (1997), 277(5324), 381-383
CODEN: SCIEAS; ISSN: 0036-8075
PB American Association for the Advancement of Science
DT Journal
LA English
AB The **yeast** cytoplasmically inherited genetic determinant [PSI+] is presumed to be a manifestation of the **prion**-like properties of the **Sup35** protein (Sup35p). Here, cell-free conversion of Sup35p from [psi-] cells (Sup35ppsi-) to the **prion**-like [PSI+]-specific form (Sup35pPSI+) was obsd. The conversion reaction could be repeated for several consecutive cycles, thus modeling in vitro continuous [PSI+] propagation. Size fractionation of lysates of [PSI+] cells demonstrated that the converting activity was assocd. solely with Sup35pPSI+ **aggregates**, which agrees with the nucleation model for [PSI+] propagation. Sup35pPSI+ was purified and showed high conversion activity, thus confirming the **prion** hypothesis for Sup35p.

L14 ANSWER 19 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1997:419850 CAPLUS
DN 127:158096
TI **Prion**-inducing domain 2-114 of **yeast** **Sup35** protein transforms in vitro into **amyloid**-like filaments
AU King, Chih-Yen; Tittmann, Peter; Gross, Heinz; Gebert, Roland; Aebi, Markus; Wuthrich, Kurt
CS Institut fur Molekularbiologie und Biophysik, Eidgenossische Technische Hochschule, Zurich, CH-8093, Switz.
SO Proc. Natl. Acad. Sci. U. S. A. (1997), 94(13), 6618-6622
CODEN: PNASA6; ISSN: 0027-8424
PB National Academy of Sciences
DT Journal
LA English
AB The **yeast** non-Mendelian genetic factor [PSI], which enhances the efficiency of tRNA-mediated nonsense suppression in Saccharomyces cerevisiae, is thought to be an abnormal cellular isoform of the **Sup35** protein. Genetic studies have established that the N-terminal part of the **Sup35** protein is sufficient for the genesis as well as the maintenance of [PSI]. Here we demonstrate that the N-terminal polypeptide fragment consisting of residues 2-114 of Sup35p, Sup35pN, spontaneously **aggregates** to form thin filaments in vitro. The filaments show a .beta.-sheet-type CD spectrum, exhibit increased protease resistance, and show **amyloid**-like optical properties. It is further shown that filament growth in freshly prepd. Sup35pN solns. can be induced by seeding with a dil. suspension of preformed filaments. These results suggest that the abnormal cellular isoform of Sup35p is an **amyloid**-like **aggregate** and further indicate that seeding might be responsible for the maintenance of the [PSI] element in vivo.

L14 ANSWER 20 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1997:270825 CAPLUS
DN 126:328816
TI Physiology and pathology of tau protein kinases in relation to Alzheimer's disease
AU Imahori, Kazutomo; Uchida, Tsuneko
CS Mitsubishi Kasei Institute Life Sciences, Machida, 194, Japan
SO J. Biochem. (Tokyo) (1997), 121(2), 179-188
CODEN: JOBIAO; ISSN: 0021-924X
PB Japanese Biochemical Society
DT Journal; General Review
LA English
AB A review with 63 refs. Alzheimer's disease (AD) is characterized by neuronal cell death and two kinds of deposits, neurofibrillary tangles (NFT) and senile **plaques**. The main component of NFT is paired helical filaments (PHF), which mainly consist of hyperphosphorylated tau protein. Tau protein kinases I and II were found as candidate enzymes responsible for hyperphosphorylation of tau to induce the formation of PHF. Since prior phosphorylation of tau by TPKII strongly enhanced the action of TPKI, it was thought that TPKII was involved in the formation of PHF-tau in concert with TPKI. After cloning, TPKI was identical with glycogen synthase kinase 3.beta. (GSK3.beta.), while TPKII consists of a novel 23 kDa protein activator and a catalytic subunit that is identical with cyclin-dependent kinase 5 (CDK5). The phosphorylation sites on tau by TPKI and TPKII could account for the most, but not all, of the major phosphorylation sites of fetal tau and PHF-tau. An antibody for a site specifically phosphorylated by TPKI (Ser413) could identify all three neurofibrillary lesions in the AD brain, and double staining for either TPKI or TPKII and NFT in the brain of Down's syndrome patients clearly demonstrated that TPKI and TPKII are both assocd. with NFT in vivo, suggesting that the level of TPKI or TPKII is elevated in AD brain by some mechanism. The levels of both TPKs change developmentally, being high in the neonatal period when the phosphorylation of fetal tau proceeds actively, suggesting that the TPKI/TPKII cooperative system has an important physiol. role in the formation of neural networks. In AD brain, aberrant accumulation of **amyloid**-.beta. protein (A.beta.) occurs ahead of the accumulation of PHF in NFT. When a primary culture of embryonic rat hippocampus was treated with 20 .mu.M A.beta., induction of TPKI, extensive phosphorylation of tau and then programmed cell death were obsd., indicating that TPKI induced by A.beta. phosphorylates tau, followed by disruption of axonal transportation and finally cell death. By using a **yeast** two hybrid system, TPKI was found to interact with pyruvate dehydrogenase (PDH), which is a key enzyme in the glycolytic pathway. PDH was phosphorylated in vitro by TPKI to reduce the activity converting pyruvate into acetyl-CoA, which is required for acetylcholine synthesis. In a primary culture of rat hippocampal cells treated with A.beta., PDH was inactivated in inverse relation to the activation of TPKI, resulting in accumulation of pyruvate or lactate, energy failure induced by the disturbance of glucose metab., and a shortage of acetylcholine owing to deficiency of acetyl-CoA, all of which are characteristic of AD brain. In cholinergic neurons such as those of the septum, non-**aggregated** A.beta., specifically A.beta. (1-42), not A.beta. (1-40), caused a shortage of acetylcholine by activation of TPKI and inactivation of PDH without cell death.

L14 ANSWER 21 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1996:685429 CAPLUS
DN 125:322366
TI Method for protein folding
IN Bohr, Jakob; Bohr, Henrik Georg; Brunak, Soeren

PA Den.
SO PCT Int. Appl., 98 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
PI WO 9630394	A1	19961003	WO 1996-DK158	19960401
W: AL, AM, AT, AT, AU, AZ, BB, BG, BR, BY, CA, CH, CN, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, HU, IS, JP, KE, KG, KP, KR, KZ, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT				
RW: KE, LS, MW, SD, SZ, UG, AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF				
AU 9653321	A1	19961016	AU 1996-53321	19960401
EP 817794	A1	19980114	EP 1996-909982	19960401
R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI				

PRAI DK 1995-361 19950331
WO 1996-DK158 19960401

AB The invention relates to the tech. application of electromagnetic radiation such as microwaves and radio waves and application of ultrasound to chain mols., e.g., biopolymers. In particular, the present invention relates to the utilization of topol. excitations such as wring, twist and torsional modes, e.g., for generating structure, such as in folding, refolding or renaturation, and denaturation or unfolding of peptides, proteins, and enzymes; for generating changes in mol. affinity; for stimulating drug receptor interactions; and for changing mol. communication. The technique is based on a new understanding of the underlying phys. phenomenon and can also be applied to other chain mols. and biol. active biomols. and tailored polymers such as glycoproteins, antibodies, genomic chain mols. such as DNA and RNA as well as PNA, carbonates, and synthetic and natural org. polymers. The invention is esp. applicable for solving problems related to inclusion bodies and **aggregation** when using recombinant DNA and protein engineering techniques. Furthermore, the invention can be utilized in therapeutic treatment and in development and prodn. of pharmaceuticals. The area of applicability includes the biotechnol. industry, food industry, drug industry, pharmacol. industry, and chem. industry and concerns, e.g., the treatment of conditions and diseases related to influenza, hepatitis, polio, malaria, borrelia, diabetes, Alzheimer's disease, Creutzfeldt Jakob disease, other **prion**-related diseases, multiple sclerosis, cataract, heart diseases, cancer, and aging.

L14 ANSWER 22 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1996:468461 CAPLUS
DN 125:163041
TI Support for the **prion** hypothesis for inheritance of a phenotypic trait in **yeast**
AU Patino, Maria M.; Liu, Jia-Jia; Glover, John R.; Lindquist, Susan
CS Howard Hughes Med. Inst., Univ. Chicago, Chicago, IL, 60637, USA
SO Science (Washington, D. C.) (1996), 273(5275), 622-626
CODEN: SCIEAS; ISSN: 0036-8075
DT Journal
LA English
AB A cytoplasmically inherited genetic element in **yeast**, [PSI+], was confirmed to be a prionlike **aggregate** of the cellular protein **Sup35** by differential centrifugation anal. and microscopic localization of a **Sup35**-green fluorescent protein fusion. **Aggregation** depended on the intracellular concn. and functional state of the chaperone protein Hsp104 in the same manner as did [PSI+] inheritance. The amino-terminal and carboxy-terminal domains of

Sup35 contributed to the unusual behavior of [PSI+]. [PSI+] altered the conformational state of newly synthesized **prion** proteins, inducing them to **aggregate** as well, thus fulfilling a major tenet of the **prion** hypothesis.

L14 ANSWER 23 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1996:152882 CAPLUS
DN 124:228885
TI Two-hybrid system as a model to study the interaction of .beta.-**amyloid** peptide monomers
AU Hughes, Stephen R.; Goyal, Shefali; Sun, Jeannie E.; Gonzalez-DeWhitt, Patricia; Fortes, MaryAnn; Riedel, Norbert G.; Sahasrabudhe, Sudhir R.
CS Neurosci. Therapeutic Domain, Hoechst Marion Rossel Inc., Somerville, NJ, 08876, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1996), 93(5), 2065-70
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB The kinetics of **amyloid** fibril formation by .beta.-**amyloid** peptide (A.beta.) are typical of a nucleation-dependent polymn. mechanism. This type of mechanism suggests that the study of the interaction of A.beta. with itself can provide some valuable insights into Alzheimer disease **amyloidosis**. Interaction of A.beta. with itself was explored with the **yeast** two-hybrid system. Fusion proteins were created by linking the A.beta. fragment to a LexA DNA-binding domain (bait) and also to a B42 transactivation domain (prey). Protein-protein interactions were measured by expression of these fusion proteins in Saccharomyces cerevisiae harboring lacZ (.beta.-galactosidase) and LEU2 (leucine utilization) genes under the control of LexA-dependent operators. This approach suggests that the A.beta. mol. is capable of interacting with itself in vivo in the **yeast** cell nucleus. LexA protein fused to the Drosophila protein bicoid (LexA-bicoid) failed to interact with the B42 fragment fused to A.beta., indicating that the obsd. A.beta.-A.beta. interaction was specific. Specificity was further shown by the finding that no significant interaction was obsd. in **yeast** expressing LexA-A.beta. bait when the B42 transactivation domain was fused to an A.beta. fragment with Phe-Phe at residues 19 and 20 replaced by Thr-Thr (A.beta.TT), a finding that is consistent with in vitro observations made by others. Moreover, when a peptide fragment bearing this substitution was mixed with native A.beta.-(1-40), it inhibited formation of fibrils in vitro as examd. by electron microscopy. The findings presented in this paper suggest that the two-hybrid system can be used to study the interaction of A.beta. monomers and to define the peptide sequences that may be important in nucleation-dependent **aggregation**.

L14 ANSWER 24 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1995:944352 CAPLUS
DN 124:27204
TI A human ubiquitin conjugating enzyme, L-UBC, maps in the Alzheimer's disease locus on chromosome 14q24.3
AU Robinson, P.A.; Leek, J.P.; Thompson, J.; Carr, I.M.; Bailey, A.; Moynihan, T.P.; Coletta, P.L.; Lench, N.J.; Markham, A.F.
CS St. James's University Hospital, University of Leeds, Leeds, LS9 7TF, UK
SO Mamm. Genome (1995), Volume Date 1995, 6(10), 725-31
CODEN: MAMGEC; ISSN: 0938-8990
DT Journal
LA English
AB The authors have identified a novel ubiquitin conjugating enzyme gene, L-UBC, which maps to human Chromosome (Chr) 14q24.3. This is also the

location of the major early onset familial Alzheimer's disease gene (FAD3). L-UBC encodes a protein that demonstrates homol. to the **yeast** ubiquitin conjugating enzyme, UBC-4, and human UbcH5. Their functions are to ubiquitinate specific proteins targeted for degrdn. The protein also exhibits very strong homol. to a rabbit protein, E2-F1, which mediates p53 degrdn. driven by papilloma virus E6 protein in vitro. The accumulation of specific proteins that have undergone aberrant processing in neurofibrillary tangles and **amyloid plaques** is the classic pathol. feature in brains of Alzheimer's disease patients. Abnormal ubiquitination has previously been suggested to play a role in the etiol. of Alzheimer's disease. This gene therefore represents a plausible candidate gene for FAD3.

L14 ANSWER 25 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1995:67677 CAPLUS
DN 122:7076
TI Overexpression of a C-terminal portion of the .beta.-**amyloid** precursor protein in mouse brains by transplantation of transformed neuronal cells
AU Fukuchi, Ken Ichiro; Kunkel, Dennis D.; Schwartzkroin, Philip A.; Kamino, Kouzin; Ogburn, Charles E.; Furlong, Clement E.; Martin, George M.
CS Department of Pathology, University of Washington, Seattle, WA, 98195, USA
SO Exp. Neurol. (1994), 127(2), 253-64
CODEN: EXNEAC; ISSN: 0014-4886
DT Journal
LA English
AB The role of .beta.-**amyloid** protein and its precursor protein is a central question in the pathogenesis of Alzheimer's disease. The authors have established several transformants from a mouse embryonic carcinoma cell line, which overproduce a C-terminal region of the .beta.-**amyloid** precursor protein from the integrated DNA constructs. These stable transformants degenerated to varying extents when undergoing neural differentiation mediated by retinoic acid. To test the neurotoxicity and the **amyloidogenicity** of the transgene product and its proteolytic derivs. in vivo, two stable transformants were neuronally differentiated and transplanted into the hippocampal regions of syngeneic mice. Similarly, either a nontransformant or a transformant bearing a cDNA construct for **yeast** major apurinic endonuclease was transplanted to the contralateral regions of the same mice. Three weeks after transplantation, grafts were identified around needle tracts or in hippocampal regions. The regions where transformants over producing the C-terminal region were grafted were highly reactive to antibodies raised against .beta.-**amyloid** protein and its precursor protein, in contrast to the contralateral regions. At 2 and 5 mo after neurotransplantation, remarkable distortion and shrinkage characterized the hippocampus on the sides injected with the transformants over producing the C-terminal region. This shrinkage was assocd. particularly with a loss of the hippocampal granule cells. .beta.-**Amyloid** protein immunoreactive granular deposits in the neuropil were also found in the same sides. Hippocampal blood vessel walls were also stained with the antibodies. These walls were surrounded by astrocytic processes, suggesting involvement of astroglial cells in vascular deposits of .beta.-**amyloid** protein. The results are consistent with the hypothesis that the C-terminal region or its derivs. are neurotoxic and **amyloidogenic**.

L14 ANSWER 26 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1994:24916 CAPLUS
DN 120:24916
TI Expression of the human .beta.-**amyloid** precursor protein gene from **yeast** artificial chromosome in transgenic mice

AU Pearson, Barbara E.; Choi, Ted K.
CS GenPharm Int., Mountain View, CA, 94043, USA
SO Proc. Natl. Acad. Sci. U. S. A. (1993), 90(22), 10578-82
CODEN: PNASA6; ISSN: 0027-8424
DT Journal
LA English
AB One hallmark of Alzheimer disease is the formation in the brain of **amyloid plaques** contg. a small peptide derived from the .beta.-**amyloid** precursor protein (APP). The APP gene exhibits a complex pattern of expression in peripheral tissues and in the brain. The entire human APP gene was introduced into embryonic stem (ES) cells by co-lipofection of a 650-kb **yeast** artificial chromosome (YAC). Three ES lines contg. an essentially intact YAC were isolated, and expression of human APP mRNAs at levels comparable to those of endogenous mouse APP transcripts was obtained. A transgenic mouse line was established by germ-line transmission of the APP YAC. RNase protection anal. of human APP mRNAs demonstrated appropriate splicing of the primary APP transcript in ES cells and in the brain of a transgenic animal. These mice may be useful for elucidating the function of the various APP isoforms in vivo.

A.beta. terminating at amino acid 40 (A.beta.x-40) was obsd. following brain injury in APP-YAC mice (P < 0.05 compared with sham control levels).
Our data show that the APP-YAC mice do not develop AD-like neuropathol. following traumatic brain injury. This may be because this injury does not induce elevated levels of the more **amyloidogenic** forms of human A.beta. (i.e., A.beta.x-42/43) in these mice.

L14 ANSWER 14 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:110408 CAPLUS
DN 128:227360
TI Saccharomyces cerevisiae Hsp104
AU Schirmer, Eric C.; Lindquist, Susan
CS Department of Molecular Genetics and Howard, Hughes Medical Institute, The
University of Chicago, Chicago, IL, 60637, USA
SO Guideb. Mol. Chaperones Protein-Folding Catal. (1997), 249-251.
Editor(s): Gething, Mary-Jane. Publisher: Oxford University Press, Oxford,
UK.
CODEN: 65RBAT
DT Conference; General Review
LA English
AB A review with 16 refs. In Saccharomyces cerevisiae, Hsp104 plays an important role in helping cells survive extreme environmental stresses such as high temps. and high concns. of ethanol. Its function in stress tolerance is related to its ability to promote the resolubilization of protein **aggregates**. In addn. to its role in stress tolerance, Hsp104 participates in the control of a **prion**-like factor known as [psi] in **yeast**.

L14 ANSWER 15 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:38052 CAPLUS
DN 128:177272
TI Hsp104
AU Glover, John R.; Schirmer, Eric C.; Singer, Mike A.; Lindquist, Susan L.
CS The University of Chicago, Chicago, IL, USA
SO Mol. Chaperones Life Cycle Proteins (1998), 193-224. Editor(s): Fink, Anthony L.; Goto, Yuji. Publisher: Dekker, New York, N. Y.
CODEN: 65MIAP
DT Conference; General Review
LA English
AB A review, with .apprx.122 refs. Topics discussed include: functional diversity of Hsp100-Clp proteins; ATPase activity and oligomerization of Hsp104; Hsp104 is a crucial thermotolerance factor in **yeast**; stress tolerance functions of the other Hsp100-Clp proteins; disassembly of protein **aggregates** underlies the stress-tolerance function; the mol. functions of other Hsp100s also dependent on a "disassembling" activity; Hsp104 controls the **aggregation** state of **Sup35**
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L14 ANSWER 16 OF 26 CAPLUS COPYRIGHT 1999 ACS
AN 1998:8875 CAPLUS
DN 128:163511
TI Long non-stop reading frames on the antisense strand of heat shock protein
70 genes and **prion** protein (**PrP**) genes are conserved between species
AU Rother, Kristina I.; Clay, Oliver K.; Bourquin, Jean Pierre; Silke, John; Schaffner, Walter
CS Institut Molekularbiologie II, Universitaet Zurich, Zurich, CH-8057, Switz.
SO Biol. Chem. (1997), 378(12), 1521-1530
CODEN: BICHF3; ISSN: 1431-6730
PB Walter de Gruyter & Co.
DT Journal